GENETICS

A PERIODICAL RECORD OF INVESTIGATIONS
BEARING ON HEREDITY AND VARIATION

Founded in 1916
by
GEORGE H. SHULL
with the cooperation of

WILLIAM E. CASTLE
EDWIN G. CONKLIN
CHARLES B. DAVENPORT
BRADLEY M. DAVIS
EDWARD M. EAST
ROLLINS A. EMERSON
HERBERT S. JENNINGS
THOMAS H. MORGAN
RAYMOND PEARL

Volume 39 July, 1954 Number 4

TABLE OF CONTENTS

APPLEYARD, R. K., Segregation of lambda lysogenicity during bacterial recombination in *Escherichia coli* K12 429

APPLEYARD, R. K., Segregation of new lysogenic types during growth of a doubly lysogenic strain derived from *Escherichia coli* K12 .. 440

HAAS, FELIX L., EDNA DUDGEON, FRANCES E. CLAYTON AND WILSON S. STONE, Measurement and control of some direct and indirect effects of X-radiation 453

DOBZHANSKY, THEODOSIUS, AND BORIS SPASSKY, Genetics of natural populations. XXII. A comparison of the concealed variability in *Drosophila prosaltans* with that in other species 472

BURDICK, ALLAN B., Genetics of heterosis for earliness in the tomato 488

ANDERSON, E. G., AND H. H. KRAMER, Translocations in maize involving chromosome 10 506

RATTY, FRANK J., Gene action and position effect in duplications in *Drosophila melanogaster* 513

PROUT, TIMOTHY, Genetic drift in irradiated experimental populations of *Drosophila melanogaster* 529

MENZEL, MARGARET Y., AND META S. BROWN, The significance of multivalent formation in three-species Gossypium hybrids 546

YANDERS, ARMON F., X-ray-induced dominant lethals in *Drosophila robusta* 558

MCQUATE, JOHN T., Chromosome loss caused by ultraviolet treatment of Drosophila spermatozoa 565

PUBLISHED BIMONTHLY AT BALTIMORE, MARYLAND
BY GENETICS, INC.

(Date of issue, August 10, 1954)

EDITORIAL BOARD

R. ALEXANDER BRINK, *Managing Editor,*
JAMES F. CROW, *Associate Managing Editor,*
University of Wisconsin

GEORGE W. BEADLE
California Institute of Technology

WILLIAM E. CASTLE
University of California

BRADLEY M. DAVIS
University of Michigan

L. C. DUNN
Columbia University

JOHN W. GOWEN
Iowa State College

DONALD F. JONES
Connecticut Agricultural Experiment Station

M. M. RHOADES
University of Illinois

GEORGE H. SHULL
Princeton University

T. M. SONNEBORN
Indiana University (Representative of the Genetics Society of America)

L. J. STADLER
U. S. Dept. Agriculture, University of Missouri

CURT STERN
University of California

ALFRED H. STURTEVANT
California Institute of Technology

SEWALL WRIGHT
University of Chicago

Volume 39 JULY, 1954 Number 4

GENETICS is a bimonthly journal issued in annual volumes of about 600 pages each.

Subscription, $8.00 net a year for complete volumes (January-November). Foreign postage, 50 cents additional. Single copies, $1.50 each, postpaid.

As available, back volumes may be had at $14.00 each and single issues of previous volumes at $2.50 per copy, postpaid. The Business Office will supply information on request as to volumes and numbers available.

Business Correspondence, including change of address, etc., should be addressed to GENETICS, INC., Business Office, Mount Royal and Guilford Avenues, Baltimore 2, Maryland.

Remittances should be made payable to GENETICS, INC.

Correspondence concerning editorial matters should be addressed to the EDITORS OF GENETICS, Genetics Building, University of Wisconsin, Madison 6, Wisconsin.

Entered as second-class matter at the postoffice at Lancaster, Pa., June 14, 1951, under Act of March 3, 1879. Acceptance for mailing at the special rate of postage provided for in the Act of February 28, 1925, paragraph 4, section 412, P.L.&R., authorized January 9, 1932. Application for re-entry at the Post Office at Baltimore, Maryland, pending.

Claims for missing numbers should be made within 30 days following their date of mailing. The publishers will supply missing numbers free only when they have been lost in the mails.

SEGREGATION OF LAMBDA LYSOGENICITY DURING BACTERIAL RECOMBINATION IN ESCHERICHIA COLI K12

R. K. APPLEYARD[1]

Kerckhoff Laboratories of Biology, California Institute of Technology, Pasadena, California

Received September 28, 1953

A CLOSE connection is known to exist between lysogenicity for the temperate bacteriophage lambda and the genetic apparatus of *Escherichia coli* K12. LEDERBERG and LEDERBERG (1953) have shown that lambda lysogenicity is closely linked to the galactose marker gal_4 in recombination from lysogenic × non-lysogenic crosses and in segregation from suitable heterozygous strains. WOLLMAN (1953) has demonstrated a close linkage between lambda lysogenicity and a galactose marker independently isolated by MONOD.

WOLLMAN has pointed out the possibility that lambda lysogenicity and the galactose marker differ from other genetic markers in that they are never transferred from the genetic donor to the genetic acceptor and thus do not play any part in genetic recombination. Our current picture of recombination in *E. coli* K12 is that it is asymmetric (HAYES 1952), the asymmetry being under the control of an infective agent denoted by F (LEDERBERG, CAVALLI and LEDERBERG 1952). In the simplest type of cross most of the genotype of any given recombinant comes from the F^- parent (LEDERBERG, CAVALLI and LEDERBERG *loc. cit.*). The genic contribution of the F^+ parent (gene donor) is small: individual characters which are not linked to a nutritional deficiency in the F^- parent appear from the F^+ parent in only a small fraction of the prototrophic recombinants. This appears to be the situation for both gal_4 and lysogenicity. However, when a strain A, gal^-F^+, is mixed with a strain B, gal^+F^-, under suitable conditions "transduction" of the F occurs and F^+ B cells rapidly begin to appear in the culture. Moreover, under suitable physiological conditions certain F^+ strains are known to behave phenotypically like F^- strains. It is therefore possible that on a mixed plate during a cross of A (gal^-F^+) × B (gal^+F^-) both A (gal^-F^-) and B (gal^+F^+) cells appear. Recombinations between these classes of cells could give rise to gal^- recombinants in which the gal^- gene was never transferred from an F^+ to an F^- cell in the normal recombination process. But in contrast to the direct recombinants which are genotypically predominantly like strain B, these gal^- recombinants arising by such an F inversion process should have genotypes which closely resemble that of strain A in respect of unselected characters. The possibility raised by WOLLMAN is therefore susceptible of experimental control in $F^+ \times F^-$ crosses if another unselected marker not linked to lysogenicity and gal_4 is available.

[1] Present address: Atomic Energy of Canada Limited, Chalk River, Ontario, Canada.

GENETICS 39: 429 July 1954.

Second Printing 1968 / University of Texas Printing Division, Austin

The experiments of LEDERBERG and LEDERBERG and of WOLLMAN restrict the possible ways in which we may suppose lambda lysogenicity to be organized in *E. coli* K12. However, we cannot be sure on the basis of lysogenic × non-lysogenic crosses alone whether lysogenicity consists of a specific allelic state of a bacterial gene, or of an additional genetic component attached to the bacterial genetic apparatus, or of many cytoplasmic particles under the partial or overriding control of a bacterial gene.

It is now possible to make artificial lysogenics for lambda mutants distinguished by their plaque morphology, as well as double lysogenics which release two different kinds of phage. If lysogenic × lysogenic crosses are made between strains which carry two such mutants, we can see whether each phage remains coupled to the galactose allele with which it is associated in the parent. If doubly lysogenic strains are crossed with non-lysogenics, we can see whether double lysogenicity is similarly associated with galactose, and whether it segregates in the same way as single lysogenicity. It will be shown below that the results of these experiments suggest that lambda lysogenicity in *E. coli* K12 consists of a single genetic component, or prophage, attached to the bacterial genetic apparatus.

MATERIALS AND METHODS

Bacteria. All bacteria used in the genetic experiments have been derived from a sensitive F^- strain C602 and a sensitive F^+ strain C112, which carry the genetic markers indicated (for bacterial genetic notation see LEDERBERG 1947):

C602: F^-; $(LTB_1)^-$, S^r, *lac*$^-$, V_6^r, $V_{1,5}^r$
C112: F^+; C^-H^-, *gal*$^-$, $V_{1,5}^r$

C602 is a streptomycin resistant T6 resistant derivative of a lambda sensitive strain C600, which has been described elsewhere (APPLEYARD 1954). C112 carries the galactose marker of MONOD. It was very kindly supplied by E. WOLLMAN and is identical with his strain 112-12. Each singly lysogenic derivative of these strains was made by incubation at 37°C with a high concentration of the appropriate lambda mutant on nutrient plates. Doubly lysogenic derivatives were made by 24-hour exposure to mixed phage in high concentration (2×10^9/ml of each mutant) in nutrient broth at 37°C.

Before using any of these strains in genetic experiments, derivatives resistant to infection with lambda (/λ or V_λ^r) were selected. (These strains phenotypically resemble the Lp_2^r strains of LEDERBERG and LEDERBERG (1953). The responsible gene in our strains is called V_λ, since it may not be identical with the LEDERBERGS' locus Lp_2.) This was done by incubation on nutrient plates with a high concentration of the strong virulent mutant lambda-v2. The use of lambda resistant strains in crosses of the type here intended has the advantage of preventing distortion of the results through infection after recombination.

Table 1 lists the bacterial strains used in the genetic experiments. ( ) denotes lysogenicity for the phages inside the brackets.

TABLE 1

*Bacterial strains used in genetic recombination.**

Source Strains (Sensitive to lambda)	
C602: F^-; $(LTB_1)^-S^r$, lac^- V_6^r, $V_{1,5}{}^r$	C112: F^+; C^-, H^-, gal^-, $V_{1,5}{}^r$
Derivatives	
C602/λ	C112/λ
C602 (λs)/λ	C112 (λs)/λ
C602 (λcl)/λ	C112 (λcl)/λ
C602 (λs, λcl)/λ	C112 (λs, λcl)/λ

*Genetic notation as in LEDERBERG, 1947.

Phages. Wild-type lambda is a typical small turbid plaque-forming temperate phage found in lysates of UV-induced wild-type *E. coli* K12. A pure line was obtained by three successive isolations of small plaques. The artificial lysogenics for lambda-s listed in table 1 were all made by infection with samples from a single high-titre stock, prepared by UV induction of a prototrophic artificially lysogenic strain.

Lambda-cl appeared as a true-breeding small clear plaque-forming mutant of lambda during experiments on the UV induction of a prototrophic defective lysogenic (APPLEYARD 1954). The artificial lysogenics for lambda-cl listed in table 1 also were made by infection with samples from a single high-titre stock very kindly supplied by M. LIEB.

Media and techniques. Stocks were grown, manipulated, and scored in nutrient broth or on nutrient plates as described elsewhere (APPLEYARD 1954). Scoring for lysogenicity was carried out by streaking or by the LEDERBERG replica technique on plates seeded with *E. coli,* strain C (BERTANI and WEIGLE 1953).

Additional media were used in bacterial crosses: liquid minimal medium: M9 + 0.5% glucose + 5γ/ml thiamine hydrochloride; minimal indicator medium: LEDERBERG's EMS medium (LEDERBERG 1949) supplemented with 5 γ/ml thiamine hydrochloride and containing 0.5% of the sugar under test; complete indicator medium: LEDERBERG's EMB medium (LEDERBERG 1949) containing 0.5% of the sugar under test; Difco " Endo " medium containing lactose. Nutritional deficiencies were scored by streaking on EMS glucose with appropriate growth supplements.

Bacterial crosses. All bacterial crosses were made on glucose minimal indicator medium and all prototrophic recombinants were first scored for galactose fermentation and lysogenicity. Some or all prototrophic recombinants in each class of interest were then purified by streaking for single colonies, rescored for prototrophy, galactose fermentation and lysogenicity, and scored for lactose fermentation, resistance to phage T6 and resistance to streptomycin.

Bacterial strains to be crossed were grown at 37°C with aeration in yeast extract medium for 24 hours. They were then diluted 1:200 into fresh medium and reincubated under the same conditions for 2¾ hours. The cultures then contained about 8.10^8 cells/ml and were at the end of the logarith-

mic phase of growth. This condition has been found (VOGT unpublished) to be optimal for recombination. The bacteria were centrifuged, suspended in buffer, recentrifuged and resuspended in buffer at twice the original concentrations. Equal quantities of the two suspensions were mixed and 0.2–0.3 ml aliquots poured with 3–3.5 ml EMS glucose agar to form a top layer on an EMS glucose plate. Control platings were made of equal aliquots of the separate bacterial suspensions and the separate suspensions were assayed by diluting and spreading on the surfaces of nutrient plates.

Using the velveteen replica technique of LEDERBERG and LEDERBERG (1952), colonies appearing on the surface of the EMS glucose plates were transferred to EMS galactose plates, and after 24 hours' incubation were scored for galactose fermentation. The EMS galactose plates were further replica tested against plates seeded with *E. coli* strain C and scored for lysogenicity. All or a suitable proportion (20 or more if possible) of the recombinants in each class of interest were then picked with a needle, transferred to 3 ml broth and at once streaked thinly with a loop on EMB galactose medium. In this way the selected recombinants are re-scored for galactose, and isolated colonies can be picked, eliminating possible mixtures. The single colonies picked were transferred to tubes containing liquid minimal medium. After 24 hours' incubation at 37°C each was restreaked as follows:

(1) Against T6 on Endo medium (score V_6 and *Lac*)
(2) On EMS galactose (check prototrophy and galactose fermentation)
(3) Against streptomycin on nutrient agar (score streptomycin resistance)
(4) On "C" plates with 15 secs. UV induction (score lysogenicity).

When necessary, as in all cases of suspected double lysogenics, a final check on lysogenicity was made by picking phage from the centre of the streak described in (4) above and replating on strain C to observe the individual phage plaques.

Resistance to lambda was scored by cross-streaking on nutrient plates with the strong virulent mutant lambda-v2.

Doubtful characters were rechecked throughout by a second streak test made from the minimal growth tube.

EXPERIMENTAL RESULTS

Bacterial markers. In the absence of lysogenicity *gal, S, lac,* V_6, and V_λ segregated as shown in table 2. No linkage was observed between any pair of characters except *lac* and V_6 (table 3). In the presence of lysogenicity no linkage has been observed between *S, lac* or V_6, on the one hand, and *gal* or lysogenicity on the other, in any of the crosses indicated in tables 2–7.

Abnormal behavior of F^- lambda resistant non-lysogenic strain C602/λ. Streptomycin resistance segregates abnormally in the cross C602/λ × C112 (table 2), 60–80% of recombinants being S^s like the F^+ parent and 96% being $V_\lambda{}^s$ like the F^+ parent. In C602 × C112 the normal behavior is observed, only 10% of the recombinants being S^s. The same abnormal segregation of *S* has been observed whenever C602/λ has been used as the F^- parent

TABLE 2

Segregation in absence of lysogenicity.

F^-; $(LTB_1)^- S^r\ lac^- V_6^r \times F^+$; $(CH)^- gal^-$

Cross	% prototrophs resembling F^+ parent for					% gal^- prototrophs resembling F^+ parent for S^s
	lac^+	V_6^s	S^s	V_λ^s	gal^-	
C602/λ × C112 at 37°	40 ± 7	42 ± 7	64 ±7	94 ±4	2.1 ±0.5	65 ±9
at 25°	42 ± 7	44 ± 7	82 ±6	98 ±2	14.3 ±1.6	64 ±6
C602 × C112 at 25°	45 ±11	35 ±10	10 ±7	....	3.1 ±0.7	10 ±7

in a cross, but not otherwise. A lethal mutation at a locus close to V_λ would cause the abnormality observed. C602/λ was therefore grown for several hours in minimal medium (M9 + glucose) so as to use up all growth factors and aliquots containing about 100 cells were spread on the surface of minimal plates (M9 + glucose, EMS – galactose) supplemented with leucine, threonine and thiamin. No colonies were formed, whereas the other derivatives of C602 formed colonies under these conditions. It was concluded that C602/λ carried a mutation for another growth factor in addition to leucine, threonine and thiamin. The abnormal segregation of S and V_λ is attributed to this mutation.

Non-allelic V_λ^r mutations. Whenever lambda resistant lysogenic F^+ strains are crossed with lambda resistant non-lysogenic F^- strains, the possibility exists that the mutations to lambda resistance of the two strains are at different loci. As a result lambda-sensitive prototrophic recombinants would be found. A possible error could then arise through transfer of lysogenicity from F^+ parent to lambda sensitive recombinants by external infection after recombination. To eliminate any possible error, in crosses of this kind samples of all significant classes of recombinants were scored for lambda resistance. No lambda-sensitive recombinants were found in any cross except those involving the F^+ double lysogenic C112 (λs, λcl)/λ. When this strain was crossed with the standard F^- non-lysogenic C602/λ, 50% of the recombinants were lambda sensitive. The same result was found with an independently occurring λ-resistant derivative of C112 (λs, λcl). It is concluded that the most common mutation to lambda resistance in C112 (λs, λcl) strains occurred in a gene not allelic with the gene which mutated to V_λ^r in our other

TABLE 3

Linkage of V_6 with lac in absence of lysogenicity.

F^-; $(LTB_1)^- S^r\ lac^- V_6^r \times F^+$; $(CH)^-\ gal^-$

Cross	% prototrophs			
	$lac^+ V_6^s$	$lac^+ V_6^r$	$lac^- V_6^s$	$lac^- V_6^r$
C602/λ × C112 at 37°	34 ± 7	6 ±3	8 ±4	52 ± 7
at 25°	40 ± 7	2 ±2	4 ±3	54 ± 7
C602 × C112 at 25°	40 ±11	5 ±5	0(0/20)	55 ±11

TABLE 4

Comparison of lambda-cI and lambda-s in lysogenic × non-lysogenic crosses at 25°C.

Cross	F^- parent $(LTB_1)^- S^r lac^- V_6^r$	F^+ parent $(CH)^-$	% prototrophs resembling F^+ parent for				% prototrophs like F^+ parent for S^s	
			——	*lys*	*gal*	*lys* + *gal*	In class like F^- parent for gal^+	In class like F^+ parent for gal^-
C602/λ × C112 (λs)/λ	gal^+ sens	gal^- (λs)	99.1 ± 0.4	0(0/2000)	0.1 (1)	0.6 ± 0.2	55 ± 11	62.5 ± 17
C602/λ × C112 (λcl)/λ	gal^+ sens	gal^- (λcl)	98.1 ± 0.3	0.1 (1)	0.2 (1)	1.0 ± 0.3	73 ± 10	57 ± 13
C602 (λs)/λ × C112/λ	gal^+ (λs)	gal^- sens	94.2 ± 0.5	0.4 (2)	0.2 (1)	5.2 ± 0.5	5 ± 5	3 ± 3
C602 (λcl)/λ × C112/λ	gal^+ (λcl)	gal^- sens	93.4 ± 0.5	0.8 ± 0.3	0(0/2000)	5.8 ± 0.5	5 ± 5	3 ± 3

TABLE 5

Effect of temperature on transfer of lysogenicity and of gal from F^+ parent to prototrophs.

Cross	F^- parent $(LTB_1)^- S^r lac^- V_6^r$	F^+ parent $(CH)^-$	% prototrophs like F^+ parent for lys		% prototrophs like F^+ parent for gal	
			25°C	37°C	25°C	37°C
C602/λ × C112 (λs)/λ	gal^+ sens	gal^- (λs)	0.6 ± 0.2	0.12 ± 0.07	0.7 ± 0.2	0.16 ± 0.07
C602/λ × C112 (λcl)/λ	gal^+ sens	gal^- (λcl)	1.1 ± 0.3	0.07 (1)	1.2 ± 0.3	0.07 (1)
C602 (λs)/λ × C112/λ	gal^+ (λs)	gal^- sens	5.6 ± 0.5	1.8 ± 0.6	5.4 ± 0.5	1.5 ± 0.5
C602 (λcl)/λ × C112/λ	gal^+ (λcl)	gal^- sens	6.6 ± 0.6	3.7 ± 0.4	5.8 ± 0.5	3.5 ± 0.4

strains. This fact will be further discussed in connection with the crosses of F^+ double lysogenic × F^- non-lysogenic.

Comparison of lambda phages. In lysogenic × sensitive crosses lambda-s and lambda-cl behave alike. Lysogenicity is closely linked to galactose: both are rarely transferred from the F^+ parent to the prototrophic recombinants (table 4). This transfer from the F^+ parent is to a considerable degree suppressed when the crosses are carried out at 37°C instead of at 25°C (table 5).

Crosses of lysogenic × lysogenic (table 6). When F^+ and F^- lysogenics, distinguished by carrying lambda-s and lambda-cl, are crossed, no double lysogenics or sensitives are found. Each mutant appears in the recombinants strongly but not absolutely linked to the galactose marker with which it entered the cross. Each is transferred about as frequently from the F^+ parent to the recombinants as is lysogenicity in F^+ lysogenic × F^- sensitive crosses.

The last two columns of table 6 show that 90–95% of recombinants are S^r like the F^- parent whether they are lysogenic like the F^+ parent or the F^-. It is therefore unlikely that lysogenicity is transferred in these crosses by F inversion, in accordance with the possibility suggested by WOLLMAN. Such an F inversion process would result in 90% S^s among those recombinants lysogenic like the F^+ parent.

Crosses of double lysogenics. When a double lysogenic is crossed with a non-lysogenic strain, the number of classes to be scored is increased by the possible appearance of single lysogenicity. Table 7 shows the behavior of double lysogenicity in such crosses.

If double lysogenicity is carried by the F^- parent, linkage with galactose is preserved. The small fraction of singly lysogenic recombinants seen is not significant, in view of the tendency of double lysogenics to throw off singly lysogenic clones during growth (APPLEYARD 1954). It has already been shown (table 4) that when F^- single lysogenics recombine with F^+ sensitive cells over 90% of the recombinants resemble the F^- parent for gal and lysogenicity. In the crosses of F^-gal^+ double lysogenic with F^+gal^- non-lysogenic, gal^- non-lysogenic recombinants are about as frequent as singly lysogenic recombinants. Most of the gal^- non-lysogenic recombinants can therefore not arise from F^- single lysogenic × F^+ non-lysogenic recombinations, but must result from recombinations between F^- double lysogenic and F^+ non-lysogenic cells.

If double lysogenicity is in the F^+ parent, the genetic transfer of lysogenicity to the prototrophic recombinants is almost completely suppressed. The transfer of the galactose marker from the F^+ parent is also much reduced. A few singly lysogenic gal^- prototrophs have been observed. These are attributed to recombinations involving single lysogenics which have arisen during the growth of the doubly lysogenic F^+ culture. Nearly all the singly lysogenic recombinants observed showed "unstable" lysogenicity, repeatedly throwing off non-lysogenic clones during growth. Pure (stable) lysogenic strains were derived from 11 of these by 4–5 single colony isolations using LEDERBERG's indirect selection technique. All were gal^+ and sensitive to lambda-v2 ($V_\lambda{}^s$). Such "unstable" lysogenics probably arise by external

TABLE 6

Segregation of lysogenicity and gal in lysogenic × lysogenic crosses at 25°C.

Cross	F^- parent $(LTB_1)^-S^r$ $lac^-V_6^r$	F^+ parent $(CH)^-$	% prototrophs resembling F^+ parent for				% prototrophs resembling F^+ parent for S^s within class	
			——	*gal*	*lys*	*gal* + *lys*	like F^- for *gal* and *lys*	like F^+ for *gal* and *lys*
C602 (λcl)/λ × C112 (λs)/λ	gal$^+$ (λcl)	gal$^-$ (λs)	97.7 ± 0.3	0.2 ± 0.1	Small, not scored	2.1 ± 0.3	10 ± 7	5 ± 4
C602 (λs)/λ × C112 (λcl)/λ	gal$^+$ (λs)	gal$^-$ (λcl)	96.8 ± 0.3	0.7 ± 0.25	Small, not scored	2.5 ± 0.3	5 ± 5	5 ± 4

TABLE 7

Segregation of double lysogenicity in crosses with sensitivity at 25°C.

Cross	F^- parent $(LTB_1)^-S^r$ $lac^-V_6^r$	F^+ parent $(CH)^-$	% prototrophs			
			gal$^+$ (λs,λcl)	gal$^-$ (λs,λcl)	gal$^+$ sens	gal$^-$ sens
C602 (λs,λcl)/λ × C112/λ	gal$^+$ (λs,λcl)	gal$^-$ sens	94.9	0.1 (1)	0	3.6 ± 0.6
C602/λ × C112 (λs,λcl)/λ	gal$^+$ sens	gal$^-$ (λs,λcl)	0	0.03 (1)	98.6	0.14 ± 0.07

Cross	gal$^+$ (λs)	gal$^-$ (λs)	gal$^+$ (λcl)	gal$^-$ (λcl)	% like F^+ parent for S^s in class	
					like F^- parent for gal$^+$	like F^+ parent for gal$^-$
C602 (λs,λcl)/λ × C112/λ	Not scored	Not scored	1.5	0	5 ± 5	0(0/39)
C602/λ × C112 (λs,λcl)/λ	0.3 (u.s.)	0	0.8 (u.s.)	0.1 (1)	70 ± 10	100 (5/5)

u.s. = unstable.

infection. They have only been observed in the crosses of F^+ double lysogenic $\times$ F^- non-lysogenic in which 50% of the prototrophic recombinants are lambda-sensitive and therefore liable to become lysogenic by external infection.

The total number of prototrophic recombinants observed in all crosses using the same F^- parent (C602/λ) was approximately the same, whether double, single or no lysogenicity was present in the F^+ parent. Throughout all other crosses discussed the number of prototrophic recombinants observed was also constant, but about ten times as great as in crosses involving C602/λ. This observation is in agreement with the hypothesis that C602/λ carries a lethal mutation not present in our other F^- strains. Within experimental error the segregation ratios of *S*, *lac* and V_6 remain constant (except as noted earlier for *S*) throughout all the crosses of this series.

DISCUSSION

Both LEDERBERG and LEDERBERG (1953) and WOLLMAN (1953) have already shown that lambda lysogenicity is allelic to non-lysogenicity and is closely linked to galactose in lysogenic $\times$ non-lysogenic crosses. They have concluded that there is a genic component of lysogenicity. The present experiments extend these studies. They show that in recombination between strains which carry distinguishable lambda mutants neither non-lysogenics, nor double lysogenics able to release both kinds of phage, are formed. The phage character which differentiates the two mutants turns out to be closely linked to galactose. Moreover when a doubly lysogenic gal^+F^- strain is crossed with a non-lysogenic gal^-F^+ strain, non-lysogenicity and gal^- are

TABLE 8

Transfer of gal^- from lysogenic and non-lysogenic F^+ parents to prototrophic recombinants in crosses at 25°C with the same non-lysogenic F^- parent (C602/λ).

Lysogenicity of F^+ parent	% gal^- recombinants
None	14.3 ±1.6
Single	0.95 ± 0.2
Double	0.14 ± 0.07

closely associated in the small percentage of recombinants in which either appears. Double lysogenicity is therefore linked to galactose just as is single lysogenicity.

The experiments also indicate (e.g., table 8) that when otherwise genetically similar F^+ strains are crossed with F^- strains transfer of lysogenicity status and galactose character from F^+ parent to recombinants is greatly reduced if single rather than non-lysogenicity is to be transferred, and still further reduced in the case of double lysogenicity. In the case of single lysogenicity this transfer is much reduced if the temperature is increased, suggesting a parallel with the work of LIEB (1953) on establishment of lysogenicity after infection.

No evidence has been found that lambda mutant characters are carried by

any hypothetical cytoplasmic particles under genic control. Further discussion will therefore be limited to the genic component of lysogenicity.

The recombination data demonstrate that this is a complex entity, for not only does it control ability or inability to release phage under suitable circumstances, but also embodies determinants of individual hereditary characteristics of the phage concerned ("prophage genes," APPLEYARD 1954). It is therefore far from a simple bacterial gene, but so far as the recombination data are concerned could be equally a complex of bacterial genes, one controlling each phage character, or an additional cellular component, the prophage, attached to the bacterial genetic apparatus at a site which could correspond to a simple bacterial gene.

It is pertinent here to suggest some other reasons for preferring the second kind of hypothesis (cf. also LWOFF 1953):

(1) It provides a more satisfactory basis for the explanation of prophage recombination (APPLEYARD 1954). The attached prophages in a double lysogenic can lie side by side. They have sufficient free ends to make interpretation even of the existing very limited data much easier.

(2) It is general experience (cf. also APPLEYARD 1954) that when a cell is cured of its lysogenicity, for example by prolonged exposure to ultraviolet light, it usually reverts to full sensitivity: it can be reinfected and lysogenized quite normally. On the basis of the gene complex hypothesis we would conclude that simultaneous reverse mutations of all the genes involved was more frequent than reverse mutation of only some of them. By contrast we would expect an additional component to become detached and lost as a single unit.

On this basis our experiments show that the genic component of lambda lysogenicity is a complex entity embodying individual prophage genes. We suggest that this entity is an additional cellular component, the prophage, attached to the bacterial genetic apparatus at or near the galactose loci of LEDERBERG and LEDERBERG ("*gal*$_4$") and WOLLMAN.

SUMMARY

Bacterial crosses between lysogenic derivatives of *E. coli* K12 carrying different lambda mutants, and between doubly lysogenic and non-lysogenic derivatives are described. The results are consistent with the view that lysogenicity consists of an additional cellular component or prophage which is attached to the bacterial genetic apparatus at a specific locus.

ACKNOWLEDGMENTS

The author is greatly indebted to M. VOGT and M. LIEB, who very kindly placed at his disposal their experience of bacterial crosses of the kind here employed and supplied some of the bacterial strains, as well as engaging in many stimulating discussions.

This work was carried out during the author's tenure of a Rockefeller Foundation fellowship at the California Institute of Technology.

LITERATURE CITED

APPLEYARD, R. K., 1954 Segregation of new lysogenic types during growth of a doubly lysogenic strain derived from *E. coli* K 12. Genetics **39:** 440–452.

BERTANI, G., and J. J. WEIGLE, 1953 Host controlled variation in bacterial viruses. J. Bact. **65:** 113–121.

HAYES, W., 1952 Analysis of the stimulating effect of UV light on genetic recombination in *Bact. coli.* K12. Nature **169:** 1017–1018.

LEDERBERG, J., 1947 Gene recombination and linked segregation in *E. coli.* Genetics **32:** 505–525.

LEDERBERG, J., L. L. CAVALLI and E. M. LEDERBERG, 1952 Sex compatibility in *E. coli.* Genetics **37:** 720–730.

LEDERBERG, J., and E. M. LEDERBERG, 1952 Replica plating and indirect selection of bacterial mutants. J. Bact. **63:** 399–406.

1953 Genetic studies of lysogenicity in *E. coli.* Genetics **38:** 51–64.

LIEB, M., 1953 The establishment of lysogenicity in *Escherichia coli.* J. Bact. **65:** 642–651.

LWOFF, A., 1953 Lysogeny. Bact. Rev. **17:** 269–337.

WOLLMAN, E., 1953 Sur le Déterminisme Génétique de la Lysogénie. Ann. inst. Pasteur **84:** 281–293.

SEGREGATION OF NEW LYSOGENIC TYPES DURING GROWTH OF A DOUBLY LYSOGENIC STRAIN DERIVED FROM ESCHERICHIA COLI K12

R. K. APPLEYARD[1]

Kerckhoff Laboratories of Biology, California Institute of Technology, Pasadena, California

Received November 22, 1953

THE experiments to be described below are most simply interpretable in terms of a new type of genetic recombination between temperate bacteriophages. This takes place between two related prophages during normal growth of a bacterial strain the cells of which carry them both. For the purpose of such a discussion it has been found necessary to refine and extend some terms in common use (JACOB, LWOFF, SIMINOVITCH and WOLLMAN 1953) and to add others.

TERMINOLOGY

When a bacterium transmits to its descendants the ability to produce bacteriophage under suitable physiological conditions, the cell is *lysogenic*. If all the descendants of the cell possess the same property, the lysogenicity is *stable:* if a significant proportion of them do not, it is *unstable*. Stable lysogenicity frequently becomes established in one or more descendants of an unstable cell. We think of this ability to release bacteriophage as determined by some specific component of the cell, called a *prophage*. Because a sensitive cell upon infection by a temperate phage may become lysogenic and later liberate identical phage, the prophage may be thought of as a stage in the life-cycle of the phage. If each cell of a lysogenic clone is potentially able to liberate a number of types of phage, we have a *poly-lysogenic* in which each cell carries several prophages. If only one prophage is carried and only one type of phage is liberated, the clone is *mono-lysogenic*.

Many hereditary character differences observed in the mature phage are transmitted as inherited differences during the growth of the corresponding clones of lysogenic bacteria. The lysogenic bacteria therefore contain hereditary determinants of these differences. In the absence of evidence to the contrary it is reasonable to suppose that these determinants are specific components, parts of the prophage. Such components will be called *prophage genes*. Where alternative forms of a phage phenotype are determined by alternative states of the corresponding prophage gene, these alternative states will here be called *prophage alleles*, by analogy with other systems.

Lysogenic clones will be described in which the prophage is present but there is a defect which almost entirely prevents production of infective phage. The defect has the following properties (*vide infra*): (1) It is reproduced

[1] Present address: Atomic Energy Project, Chalk River, Ontario, Canada.

from generation to generation in all cells of the clone. (2) It is associated with the prophage rather than with the bacterial residuum of the lysogenic complex. (3) It is not a general malformation of the whole prophage structure but is to some extent localized. Genes carried by a defective prophage in a double lysogenic can recombine with other genes during vegetative phage development and can segregate from the defect during bacterial growth. Moreover the defect by no means impairs the whole physiological activity of the prophage.

These properties will be taken as sufficient to establish the defect as due to a gene of the prophage although the property, in this instance, is not transmissible through the infective particle stage of the temperate bacteriophage life-cycle. In default of more detailed evidence the defect will here be ascribed to a particular allele of a prophage gene. However, the cause could equally be a small or large loss of material or a modification in part of the prophage structure or of its relation to the bacterial residuum. Any of these would be consistent with the data and interpretations to be presented below.

In some poly-lysogenic clones each cell is potentially able to liberate more than one unrelated type of phage. Other clones are found in which each cell is able to liberate more than one of several closely related types of phage which differ among themselves only in a limited number of genes. These two kinds of poly-lysogenic clone will be distinguished respectively as *allolysogenic* and *homolysogenic*.

The question arises, are the prophage genes in a homolysogenic cell organized into sets, each set associated with the liberation of one of the related types of phage? If this is the case, we may properly think of each set as the gene complement of a separate prophage, whether or not there also exist cellular components which determine the over-all capacity of the cell to liberate bacteriophage. Such a usage adheres to the idea of a prophage as a continuing form of one phage. All present evidence suggests that prophage genes in a homolysogenic are, in fact, organized into separate prophages. This description of lysogenicity will therefore be taken as a basis for discussion.

The possibility exists of the interchange of prophage genes between two related prophages carried in one cell. The new combinations of prophage genes so formed would represent new types of lysogenicity. This phenomenon will be called *prophage recombination* by analogy with other systems.

EXPERIMENTAL MATERIAL

Associated with the naturally occurring strain *Escherichia coli* K12 is a temperate bacteriophage lambda, to which some natural and artificially derived strains of *Escherichia coli* are sensitive (LEDERBERG and LEDERBERG 1953). Other strains, resistant to lambda but sensitive to the strong virulent mutant lambda-v2, have been designated immune-1 by LEDERBERG and LEDERBERG (1953). Two genetically different strains carrying the immune-1 property, which turned up in our experiments, have proved on further investigation to be lysogenic, although a defect in the prophage caused the phage yield to be

very small. Such strains will be called *defective lysogenics*. When one of these strains was superinfected with the weak virulent mutant lambda-v1, a homolysogenic clone arose. During growth this homolysogenic strain threw off several kinds of segregant clone which were mono- or poly-lysogenic and possessed combinations of prophage genes different from those in either the original homolysogenic or its parent defective lysogenic strain. These new combinations are interpreted as having arisen through prophage recombination.

MATERIALS AND METHODS

Bacteria. Bacteria used are listed in table 1, with the genotype and source or manner of derivation of each. The source strain Y-70 was kindly supplied by E. WITKIN. The sensitive strain K12S was kindly supplied by E. M. LEDERBERG. At that time it possessed a second nutritional requirement which was lost during subsequent growth.

Phages. Table 2 describes the lambda phages which have been used and their sources. Lambda-v2 was kindly supplied by E. M. LEDERBERG. The remaining mutants were isolated and kindly supplied by M. LIEB. All temperate phage stocks were repurified by the repeated picking of single plaques. High titre phage stocks were then made by ultraviolet induction of corresponding lysogenic strains.

Media. (a) Culture media as described by WEIGLE and DELBRÜCK (1951).

(b) Endo medium: commercial (Difco) Endo with the addition of 0.5% NaCl and approximately 0.001% $MgSO_4 \cdot 7\ H_2O$. The exact optimal quantity

TABLE 1

Description and sources of bacterial stocks.

Stock No.	Description	Source
Lysogenic		
E. coli K12 C6	$L^-T^-B^-_1\ Lac^-V^r_{1,5}$ (λ)	Y70 (WITKIN) by selective action of T1
E. coli K12 C60	$L^-T^-B^-_1\ Lac^-V^r_{1,5}$ (λw)	C6 by selective action of lambda-v1
E. coli K12 C655	$L^-T^-B^-_1\ Lac^-V^r_{1,5}$ (λw, λv1)	C60 by action of lambda-v1
Sensitive		
E. coli K12S	B^-_1	LEDERBERG (W1294)
E. coli K12 C600	$L^-T^-B^-_1\ Lac^-V^r_{1,5}$	C60 by 2 min. UV
E. coli K12 C47	$B^-_1\ Lac^-$	Recombinant in lysogenic × sensitive cross
E. coli 122	Wild type	London Nat'l Type Culture Collection

Other Strains: **"/λ" derivatives of certain of above, derived by selective action of lambda-v2. These strains do not adsorb lambda or any of its mutants.**

L^-: leucine requiring
T^-: threonine requiring
B^-_1: thiamine requiring
Lac^-: non-lactose fermenter
$V^r_{1,5}$: resistant to coliphages T1 and T5
(λw): Lysogenic for defective modification of lambda prophage

TABLE 2

Lambda phage stocks.

Stock	Description	Source
Lambda	Temperate; forms turbid plaques; unable to plate on Endo	Principal constituent of wild-type lambda stock
Lambda-1	Temperate; forms turbid plaques; able to plate on Endo	Mutant selected from wild-type stock
Lambda-v1	Weak virulent; forms clear plaques; able to plate on Endo	Spontaneous occurrence as mutant in lambda-1 stock
Lambda-v2	Strong virulent; forms clear plaques; able to plate on Endo; able to plate on bacterial strains lysogenic for lambda	LEDERBERG'S lambda-2

of magnesium to be added depends slightly on the conditions of autoclaving and storage of the medium. A supplement of 5 γ/ml thiamine hydrochloride is added if thiamine deficient strains are to be grown. This medium deteriorates after a few days' storage. It is best used after storage for 24 hours at 4°C.

Phage assays. Phage assays are made by the agar-layer technique described by ADAMS (1950).

Irradiations with ultraviolet. These are carried out as described by WEIGLE and DELBRÜCK (1951), but at an intensity of 1.5×10^3 ergs/sec/cm^2, sufficient to kill 99% of T2 phage in 8 secs. All irradiations are carried out either in buffer or on the surface of agar plates.

Replica and seeded plate techniques. (a) The velveteen replication method of LEDERBERG and LEDERBERG (1952) has been used in testing for presence and type of lysogenicity. Seeded plates are prepared by pouring on a 1% nutrient agar plate 2.5 ml of 1.5% nutrient agar to which has been added 0.3 ml of a fresh fully grown broth culture of indicator bacteria. The plates may then be stored at 4°C for several weeks without deterioration. Test colonies are transferred to the surface of the plate by a velveteen intermediate, and the plate is exposed to ultraviolet for 10–20 secs. before incubation. Lysogenic colonies are surrounded by a clear halo after 24 hours at 37°C. The type of lysogenicity may frequently be inferred from inspection of the halo.

(b) Endo medium as "host-range" indicator: ability to plate on Endo distinguishes the mutant lambda-1 from wild type lambda more critically than plaque size, the original criterion. Lambda-1 plates on Endo with a variable efficiency in the region 20–40%. Under the same conditions the plating efficiency for wild type lambda is about 0.001%, and those phage particles which form plaques subsequently breed true for this character. Lambda-v1 and lambda-v2 are both able to plate on Endo medium with efficiencies as high as that of lambda-1. Throughout the present work the slightly variable behavior of the indicator medium has been checked with the same two standard stocks of lambda and lambda-1. The choice of bacterial indicator strain is unimportant, and the *lac*$^-$ sensitive strain C47 has usually been employed purely for convenience in visibility. To determine the plating efficiency

of a given phage stock on the medium, parallel assay plates are made on Endo and nutrient agar plates. To test the ability of lysogenic colonies to produce phage capable of plating on Endo, parallel replicas are made using the two media. The same seeded plates as are used in replication tests may be used to study the Endo plating character of the phage in a single plaque or in the replica halo surrounding a given colony. The plaque or halo is stabbed with a platinum needle bent at 90° about 1 cm from the tip. The needle is first touched to a seeded Endo plate, then to the surface of a seeded nutrient plate, which serves to check the presence of adequate phage on the needle and may also be examined to score the phage as a clear or turbid plaque former.

EXPERIMENTAL RESULTS

Derivation and properties of a defective lysogenic. The defective lysogenic strain C60 was an apparently non-lysogenic survivor after superinfection of C6 (lysogenic for wild type lambda) with the weak virulent mutant lambda-v1, followed by 4 hours' growth in broth. The experiment was suggested by the fact that such superinfection is known in Shigella to give rise to some non-lysogenic survivors (BERTANI 1953). Wild type lambda, lambda-1, and lambda-v1 plate on C60 with efficiencies less than 10^{-8} whereas lambda-v2 plates on it with the same efficiency as on sensitive or lysogenic strains of *E. coli* K12. C60 was therefore initially classified as immune-1. The following properties show that it is a defective lysogenic: (1) The sensitivity to ultraviolet killing, shown in figure 1, is at least as great as that of the parent lysogenic strain C6 under the same conditions. It is much greater than the ultraviolet sensitivity of a derived lambda-sensitive strain (C600). C600 was, however, selected as a survivor of C60 following a very heavy exposure to ultraviolet (*vide infra*). (2) After ultraviolet irradiation, the optical density drops at the same time and to much the same extent as in the parental lysogenic strain (fig. 2). Under the microscope, a similar pattern of lysis is then observed in many cells of cultures of either strain. (3) When the cells have been grown in nutrient broth to a density of 3×10^8/ml, at most one bacterium in 10^5 yields infective wild type lambda phage following ultraviolet irradiation. The average number of infective phage particles liberated per yielder bacterium is low (10–20), and the proportions of yielders increases slowly with doses up to 25 secs. In normal lysogenic strains under similar conditions 200–400 phage per bacterial yielder are observed, with a maximum proportion of yielders at a rather lower dose (10–15 secs.).

KELLENBERGER (unpublished experiments; the author is indebted to DR. E. KELLENBERGER of Geneva for permission to quote these results) has shown in electron micrographs that most cells of C60 do not liberate any particles of a size comparable to that of infective phage during lysis after UV induction.

To determine whether the defect of the defective lysogenic system resided in the prophage or in the bacterium, a sensitive strain C600 was derived by exposure of C60 to 2 min. ultraviolet irradiation. 95% of the survivors were sensitive to temperate lambda. (This experiment and the derivation of strain

C600 were carried out by Mr. A. D. Kaiser, to whom the author is indebted for permission to quote the results.) All available lambda mutants form plaques with the same efficiency on K12S and on the sensitive strain thus selected, and temperate lambda mutants form stable lysogenics. The latter

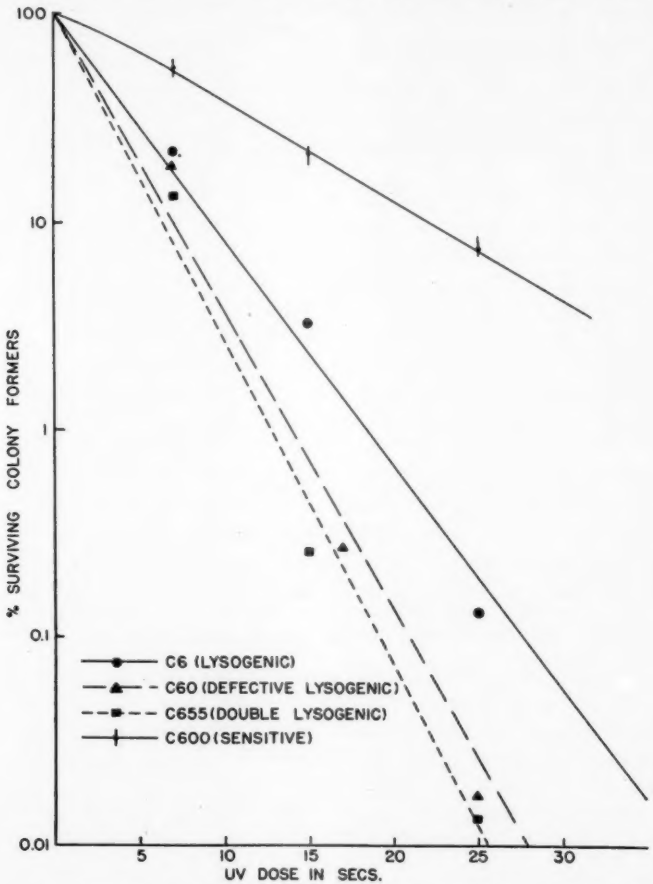

Figure 1.—UV killing of defective lysogenic strain C60 of *E. coli* K12 and related strains. Exp.: all strains were grown and irradiated as described in table 3. Assay for colony formers on nutrient plates 10 min. after irradiation.

strains are typical fully inducible lysogenics. When grown to 3×10^8 cells/ml and irradiated 10–15 secs. with ultraviolet 90% of the cells liberate phage in an average yield 200–400. It is concluded that in the defective lysogenic the defective element is the prophage. The phage liberated by C60 is wild type lambda, forming turbid plaques but unable to plate on Endo medium. Similar

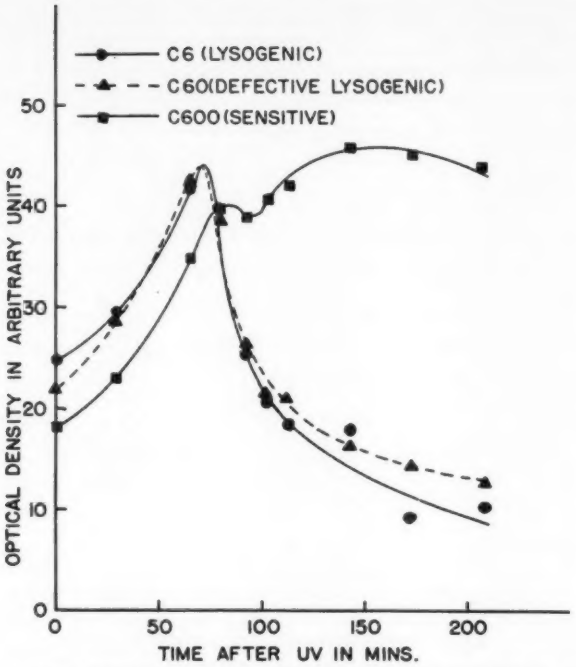

FIGURE 2.—UV induced mass lysis of defective lysogenic strain C60 of *E. coli* K12 and related strains. Exp.: cultures grown as described in table 3 were centrifuged and resuspended in an equal volume of buffer, then exposed to 15 secs. ultraviolet. They were diluted 1:1 with fresh broth at time zero and incubated at 37°C with aeration.

results have been observed with a defective lysogenic derived from wild type K12 by the action of 2 mins. ultraviolet irradiation.

Superinfection of defective lysogenic. When C60 is exposed to the weak virulent mutant lambda-v1 at a high multiplicity (10–20), the phage is adsorbed normally. About ⅔ of the cells are killed. If the infected cells are exposed to anti-lambda serum to remove free phage and so prevent reinfection, and then grown in aerated broth at 37°C for several hours, some libera-

TABLE 3

Classification of phage yield from poly-lysogenic C655.

Phage phenotype	++	+ cl	E +	E cl
No. observed in yield	8	20	14	17

E: able to plate on Endo
+: unable to plate on Endo
cl: clear plaque former
+: turbid plaque former

Expt.: 24-hr. broth culture diluted 1:200 in fresh broth and grown with aeration to $3 \cdot 10^8$ cells/ml. Diluted 1:100 in buffer and exposed 15 secs. to ultraviolet. Diluted 1:100 in fresh broth and incubated 2 hours at 37° C. Assay on nutrient plates.

tion of weak virulent lambda is observed during the first 2½ hours. After this time, few additional virulent lambda particles are produced. A very much larger number of temperate lambda are now liberated at an increasing rate at least up to 5 hours after the original infection. The surviving cells may be plated and the colonies tested for normal, i.e., non-defective, lysogenicity by replication. When plated immediately after infection, 4% of the survivors gave rise to halos on the replica plates and were classed as lysogenic. At the end of 5 hours' growth in broth, one colony out of 1200 tested was lysogenic. The pure strain (C655) derived from this colony turned out to be homolysogenic. Its behavior is discussed in the following section.

PROPERTIES OF HOMOLYSOGENIC STRAIN C655

Homolysogenicity. C655 was obtained in pure culture by three successive single colony isolations on nutrient agar plates. A stock culture was thereafter maintained on nutrient agar slants. To demonstrate the homolysogenicity a suitable broth culture was induced by ultraviolet, incubated at 37°C for 2 hours to allow the cells to lyse, and assayed for phage. Individual plaques picked at random from the assay plates were scored for clear or turbid plaque formation and for ability or inability to plate on Endo. Table 3 shows that four closely related types of phage were liberated by the induced culture in approximately equal numbers.

Effect of ultraviolet irradiation. Ultraviolet irradiations of C655 and related strains at various doses were carried out under identical conditions of growth. The bacteria were assayed on nutrient plates for surviving colony formers. The lethal effects of the ultraviolet on C655 and on the parent defective lysogenic strain C60 were found to be the same; the lethal effect of

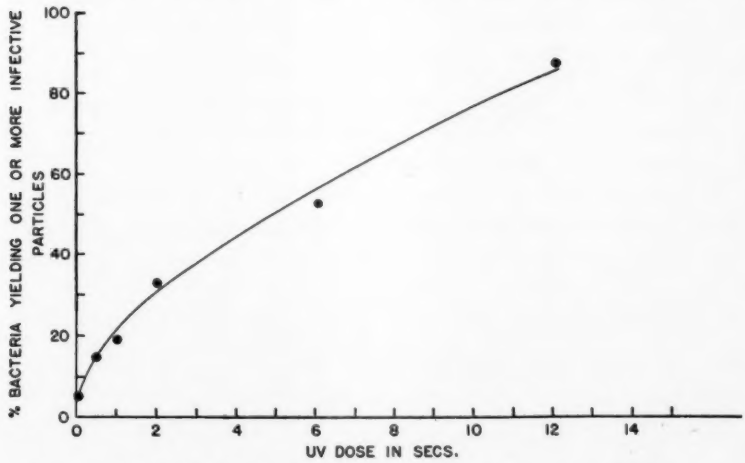

FIGURE 3.—UV induction of poly-lysogenic strain C655. Exp.: as in figure 1.

ultraviolet on the sensitive strain C600 derived from C60 was considerably smaller (fig. 1).

When a C655 culture is exposed to different doses of ultraviolet and assayed for infective centers by the agar layer technique, plaques arise either from induced cells, or from surviving colony formers if one or more spontaneous liberations of phage take place early in the colony. Under the microscope the latter type of plaque, even if turbid, is readily distinguishable by the presence at or near its center of the large single parental colony. By subtraction of this class the number of cells induced at each ultraviolet dose can be calculated. The ultraviolet induction curve of figure 3 was derived in this way. It is similar to the induction curve for wild type monolysogenics.

Yields from single induced C655 cells were examined after exposure to ultraviolet by dilution of a culture into separate tubes, each of which contained on the average 0.3 bacteria before burst. After 2 hours incubation at 37°C the contents of each tube was assayed for infective centers. 90% (27/30) of those tubes whose contents gave rise to plaques contained a mixture of clear and turbid plaque formers. The production of mixed phage yields is therefore a property of most individual ultraviolet-induced cells of C655.

Bacterial segregants from poly-lysogenic strain C655. A fresh broth culture of C655 containing 2×10^8 cells/ml was diluted and plated on nutrient agar. When the colonies were tested for lysogenicity by replication and 15 secs. ultraviolet induction, some appeared to be non-lysogenic and did not form halos, while others were distinguished by unusually turbid halos. A number of segregants of each type were obtained in pure culture by three successive single colony isolations and maintained as stocks on nutrient agar slants. The lysogenicity of each segregant was examined in the following manner. A fresh broth culture of 3×10^8 cells/ml was assayed by dilution and spreading on nutrient plates. A suitable aliquot was suspended in buffer, induced by exposure to 15 secs. ultraviolet, and incubated for 2 hours in broth at 37°C. After dilution the phage yield was assayed in parallel on nutrient plates and on Endo medium. The bacterial assay plates were replica-tested to see whether the strain threw off any further non-lysogenic segregants. Each segregant was now scored for its prophage content as follows:

Phage yield much greater than 1 per bacterium.	At least one non-defective prophage.
Phage yield much less than 1 per bacterium.	No non-defective prophage.
Phage yield all clear plaque formers.	No prophage gene for turbid plaque formation.
Phage yield all turbid plaque formers.	No prophage gene for clear plaque formation.
Phage yield mixed turbid and clear plaque formers.	Prophage genes for both turbid and clear plaque formation. Segregant is poly-lysogenic.
Less than 5% able to plate on Endo medium.	No prophage gene for ability to plate on Endo.

More than 5% able to plate on Endo medium.	At least 1 prophage gene for ability to plate on Endo.
Less than 1/10th as many apparently non-lysogenic segregants as from C655.	No defective prophage.

In the cases of some poly-lysogenics, individual phage particles from the yield were tested on Endo by the needle technique in order to show that the prophage allele for inability to plate on Endo medium was present as well as the prophage allele for ability to plate.

Table 4 summarizes the properties of the phage yields from segregant strains which have been investigated in this way and lists the totality of prophage genes present in each segregant. For convenience, the prophage genes attributed to the parental defective lysogenic and to the super-infecting phage are also listed in the table. It will be seen that all the segregant bacteria are derived from the poly-lysogenic parent C655 by the loss of one or more prophage alleles. The presence of the mutant bacterial characters listed in table 1 for strain C6 has been confirmed for each of the bacterial strains in table 4. Between the observation of bacterial segregants and the last previous single colony isolation, strain C655 divided approximately 70 times. The relative frequencies of appearance of different types of segregants might therefore have been subject to modification by selection. If, however, the bacterial segregants arise by any kind of random event, the majority of them will still be distributed in small clones of less than 50 cells. The effect of selection will therefore be small. Table 4 then shows that simultaneous loss of an allele from

TABLE 4

Bacterial segregants from poly-lysogenic C655.

Type stock	Absolute number tested	Proportion in culture %	Phage types observed in yield	Presence of weak or non-lysogenic segregants	Prophage alleles present in stock
C655			++,+cl,E+,Ecl	+	E/+,cl/+,w/+
C655 t1	12	3.6	E+	<0.1%	E,+,+
C655 t7	2	0.6	++,E+	+	E/+,+,w/+
C655 t15	1	0.3	++,+cl,E+,Ecl	<0.1%	E/+,cl/+,+
C655 i1	5	1.3	++		+,+,w
C655 i2	7	1.8	E+		E,+,w
C655 i5	1	0.3	++,+cl,E+,Ecl		E/+,cl/+,w
C60			++		+,+,w
Lambda-v1			Ecl		E,cl,+

Phage types: notation as in table 3

Prophage alleles: E: yields some phage able to plate on Endo
+: yields some phage unable to plate on Endo

cl: yields some clear plaque formers
+: yields some turbid plaque formers

w: very small phage yield following UV induction
+: high phage yield following UV induction

each of three prophage genes is a decidedly more probable event than loss of one.

A similar general pattern of mixed phage yields and of bacterial segregants has been observed using a lambda resistant derivative (C655/λ) of C655 unable to adsorb lambda phage. Such resistant strains may be selected as survivors following exposure to the strong virulent mutant lambda-v2.

DISCUSSION

Defective lysogenics. LWOFF and SIMINOVITCH (1951) have described a defective lysogenic strain of *B. megatherium* which is formally very similar in its properties to C60. Further analysis by SIMINOVITCH (1951) has shown that the defect in their strain involves an inability to resume manufacture of DNA after this process has been interrupted by ultraviolet induction. The existence of defective lysogenics in the system lambda–*E. coli* K12 has also been recognized by LEDERBERG and LEDERBERG (1953).

From each of our defective lysogenics, normal sensitives can be produced by a single massive dose of ultraviolet: the defect in the lysogenic complex is lost at the same time as the prophage. This suggests that the defect is connected with the specific prophage structure, rather than with the bacterial residuum of the complex.

JACOB (1952) has obtained a number of lysogenic strains which differ very widely in their ability to produce phage, by infection of a sensitive strain of *Pseudomonas pyocyanea.* Without further analysis it is not certain whether these variations are due to genetic heterogeneity in the bacteria or the phage used, or in the relations between them. JACOB's results differ from ours in the high frequency of occurrence of the defective strains, in the existence of a continuous gradation of intermediate degrees of defect, and in the fact that the strains were prepared by infection. It may therefore be questioned whether the same underlying cause is responsible.

By superinfection of the defective lysogenic C60, a homolysogenic has been derived which is phenotypically non-defective, but from which both defective and non-defective lysogenics segregate. Both the defective and non-defective properties of lysogenicity are therefore represented in the homolysogenic. The same homolysogenic also contains two alleles of each of two prophage genes, one controlling plaque type and the other Endo plating ability. From it, mono-lysogenics segregate which have lost one allele of each gene. In all cases so far examined, these mono-lysogenics have also lost either the defective or the non-defective property of the homo-lysogenic. It is also observed that most but not all of the segregants which have lost either the defective or the non-defective property have also lost one allele of each of the two prophage genes and are mono-lysogenic. This correlated reduction is consistent with the idea that the defect in the parental strain C60 is due to an allele of a prophage gene.

Superinfection. For the phage P2 active on Shigella, BERTANI (1953) has described the initial multiplication of a weak virulent phage which superinfects

a related lysogenic, followed by a large yield of recombinant types of phage. Our results are consistent with these. Bertani found some sensitive bacterial survivors of the superinfection process, as well as unstable lysogenics for the superinfecting phage.

Poly-lysogenicity and prophage interaction. The release of four related bacteriophages by induced C655 requires that the strain be doubly homolysogenic. A higher multiplicity for the poly-lysogenicity need not be assumed, since phage recombination does occur in the vegetative phase in this system (A. D. KAISER personal communication). In the double lysogenic one prophage carries defective lysogenicity while the other does not. Most cells of a culture are phenotypically non-defective: they yield infective particles after a suitable dose of ultraviolet irradiation. Physiological interaction between the developing prophages therefore leads to a " dominance " of the non-defective characteristic.

Prophage recombination. During growth of our poly-lysogenic, bacterial clones segregate which have lost one or more prophage alleles. A striking feature of the segregation is that in the majority of the segregants one allele of each of three prophage genes has been lost and the segregant is monolysogenic. Three different types of mono-lysogenic segregant have been observed. A minority of segregants remain poly-lysogenic but have lost a single allele of one prophage gene. Three different types of such poly-lysogenic segregant have been observed. No segregants have yet appeared in which two prophage alleles have been lost.

Among the bacterial segregants a sufficient number of different kinds occur to make it certain that some at least contain new combinations of prophage genes, i.e. combinations not present in either prophage of the parent cell of the clone. The same is true of segregants from the derived stock C655/λ which cannot adsorb lambda phage. The phenomenon is therefore not a consequence of successive cycles of prophage loss and superinfection (BERTANI loc. cit.) coupled with intervening phage recombination in the vegetative phase. While there is as yet not enough evidence for the definitive exclusion of all other alternatives, the appearance of new combinations of prophage genes during growth of a polylysogenic clone strongly suggests the following as the simplest interpretation. There exists a process, formally akin to somatic crossing over, by means of which the genes of two related prophages carried in one cell can recombine. This process, here called prophage recombination, gives rise to segregant poly-lysogenics which, as compared to the parent strain, have lost one or more prophage alleles. It is possible to account for all the observed segregants in this manner, since present experimental methods cannot distinguish a mono-lysogenic from a " homozygous " poly-lysogenic. However, because of the high frequency with which mono-lysogenic segregants occur, it is equally likely that another process, the loss of one prophage, also takes place during the growth of the poly-lysogenic.

SUMMARY

1. Two genotypically different strains of defective lysogenic *Escherichia coli* have been selected from derivatives of *Escherichia coli* K12 and shown to carry a defective lambda prophage.

2. By superinfection of one of these strains with a weak virulent mutant, lambda-v1, a poly-lysogenic strain has arisen. This carries a total of six recognizable prophage alleles. Some properties of the poly-lysogenic are described.

3. During growth of this poly-lysogenic, segregant bacterial clones arise in which one or three prophage alleles have been lost, leading to new types of mono- or poly-lysogenesis. It is suggested that this phenomenon results from a recombination of prophage genes during growth.

ACKNOWLEDGMENTS

The author is indebted to J. J. WEIGLE and M. DELBRÜCK for many stimulating and helpful discussions.

This work was carried out during the author's tenure of a Rockefeller Foundation fellowship at the California Institute of Technology.

LITERATURE CITED

ADAMS, M. H., 1950 Methods of study of bacterial viruses in "Methods in Medical Research," Vol. 2, The Year Book Publishers, Chicago.

BERTANI, G., 1953 Infections Bacteriophagiques Secondaires des Bacteries Lysogènes. Ann. Inst. Pasteur **84:** 273–280.

JACOB, F., 1952 Effets de la Carence Glucidique sur l'Induction d'un *Pseudomonas pyocyanea* Lysogène. Ann. Inst. Pasteur, **82:** 433–455.

JACOB, F., A. LWOFF, A. SIMINOVITCH and E. WOLLMAN, 1953 Definitions de Quelques Termes Relatifs a la Lysogenie. Ann. Inst. Pasteur **84:** 222–224.

LEDERBERG, E. M., and J. LEDERBERG, 1953 Genetic studies of lysogenicity in *E. coli*. Genetics **38:** 51–64.

LEDERBERG, J., and E. M. LEDERBERG, 1952 Replica plating and indirect selection of bacterial mutants. J. Bact. **63:** 399–406.

LWOFF, A., L. SIMINOVITCH and N. KJELDGAARD, 1950 Induction de production de phage dans une bactérie lysogène. Ann. Inst. Pasteur **79:** 815–858.

LWOFF, A., and L. SIMINOVITCH, 1951 Induction de la lyse d'une bactérie lysogène sans production de bacteriophage. Compt. rend. **233:** 1397–1399.

SIMINOVITCH, L., 1951 Relation entre le developpement abortif du prophage chez B. Megatherium 91(1) et la synthese de l'acide désoxyriboneucléique. Compt. rend. **233:** 1694–1696.

WEIGLE, J. J., and M. DELBRÜCK, 1951 Mutual exclusion between an infecting phage and a carried phage. J. Bact. **62:** 301–318.

MEASUREMENT AND CONTROL OF SOME DIRECT AND INDIRECT EFFECTS OF X-RADIATION

FELIX L. HAAS,[1] EDNA DUDGEON,[2] FRANCES E. CLAYTON AND WILSON S. STONE

Genetics Laboratories, Department of Zoology, University of Texas

Received November 25, 1953

DURING the first two decades following MULLER's (1927) proof that irradiation induced genetic changes, most of the effect was considered to result from ionizations (and excitations) within the chromosome itself. LEA (1946) interpreted the evidences accumulated up to that time as indicating that most genetic damage was induced directly, although he believed that ionizing and ultraviolet radiations produced also indirect effects on other components of the cells, which contributed a small part of the total effect. Such a view is no longer tenable. THODAY and READ (1947) showed that the frequency of chromosomal abnormalities induced by X-rays in *Vicia faba* was several times as great in air as it was in nitrogen for the same dosage. KOTVAL and GRAY (1947) concluded that about one-half of the effect of alpha radiation in breaking prophase chromosomes of Tradescantia must be due to energy absorbed in the medium surrounding the chromosome. That same year STONE, WYSS and HAAS (1947) demonstrated that the irradiation of the substrate with ultraviolet light, prior to growing bacteria in the irradiated medium, increased the mutation rate in these microorganisms. WYSS, STONE and CLARK (1947) showed that a similar increase in mutation rates in microorganisms could be accomplished by adding H_2O_2 (one of the active radicals produced in water by ultraviolet, or by X-rays if oxygen is present) to the substrate prior to inoculation.

These investigations provided both inferential and direct evidence that radiation-induced chemical changes occurring outside the chromosomes may subsequently induce genetic changes. The discovery of these radiation-induced chemical mutagens may permit a comparison of the effect of radiation with chemical mutagens, such as those described by AUERBACH and ROBSON (1946).

The present study is an investigation of the effect of changing the environment of mature sperm during irradiation on the production of chromosome rearrangements. The results were reported briefly (HAAS, DUDGEON, CLAYTON and STONE 1952).

MATERIALS AND METHODS

Drosophila virilis was the test organism employed for these experiments. The normal stock was established from a strain collected in Texmelucan,

[1] Now at Bristol Laboratories, Syracuse, New York.

[2] Now at Department of Biology, University of Mississippi, University, Mississippi.

Mexico (U.T. Stock No. 1801). The multiple mutant stock used was synthesized from various laboratory mutants, and contained the genes broken (*b*, 188.0) on the second chromosome, tiny bristle (*tb*, 104.0) and gapped (*gp*, 118.5) on the third, cardinal (*cd*, 32.2) on the fourth, and peach (*pe*, 203.0) on the fifth chromosome (CHINO 1941).

A plastic chamber, which held the flies at a constant temperature for sufficiently long periods of time, was used for gaseous treatment and irradiation. The chamber was so constructed that the gaseous atmosphere around the flies was continually being replaced by fresh gas at the same temperature as that of the chamber. Flies to be irradiated were placed in this chamber and held for one hour in the gas mixture at the temperature to be used during the irradiation to insure that they were in equilibrium with the gas. The chamber was then moved into position on the X-ray machine, and the flies were irradiated. After irradiation the gas and temperature treatments were continued for twenty to thirty minutes before removing the flies to a food vial. All testing and growing of Drosophila cultures was carried out in standard U.T. yeast enriched food at $23 \pm 1°C$.

For all tests with the fast dose a 220 kv Westinghouse Constant Potential X-ray machine, operating at 200 kv and 15 ma and having inherent filtration equivalent to 0.25 mm aluminum, was used for irradiation. The fly chamber was placed in position 15.4 cm from the center of the X-ray tube and was held in an apparatus so constructed that the same position was automatically assumed in every experiment. At this distance the output of the machine was 1867 r per minute within the chamber in which the flies were present. The output was checked with the aid of Sievert Ionization Chambers several times during the course of these experiments and the results were always uniform. The time of exposure to the X-radiation was regulated so as to give the total X-ray dose desired.

In several experiments the " slow dose " was administered on the same or a similar machine, in others, on a Victor machine operating at 50 kv and 5 ma with a 1 mm aluminum filter to give about 100 r per minute. As *Drosophila virilis* matures slowly, eight-day-old males were irradiated in the tests.

Most of the gases employed were specially prepared and analyzed mixtures obtained from the Houston Oxygen Company. One was a mixture of 96% nitrogen and 4% oxygen, another of 95.8% carbon dioxide and 4.2% oxygen, and the third had 99.5% oxygen. In preliminary experiments carbon monoxide was generated by heating a mixture of oxalic and sulfuric acids and collected over water. Carbon dioxide was removed to a great extent by bubbling the gas several times through sodium hydroxide solutions. In later experiments carbon monoxide obtained from the Matheson Company was used. The analysis of this gas showed 96.8% CO, 0.36% CO_2, 0.97% H_2, 1.0% N_2, 0.8% saturated hydrocarbons, and traces of iron and sulfur. This gas was mixed over water with the proper amount of CO_2 and/or O_2 to give the desired gas mixture.

Translocations were detected genetically through linkage of genes known

to be located in different linkage groups. The rare translocations which gave very viable aneuploids were not detected. Normal males were irradiated and mated individually within one or two days to several females of the multiple mutant marker stock. The male was discarded three days after mating. A group of ten to twelve F_1 males from each P_1 male were backcrossed individually to several marked females, and translocations were scored by observing the segregations in their backcross progeny. In a number of experiments some of the translocations detected genetically were examined cytologically in the salivary gland nuclei of the second backcross generation. In all doubtful cases, as well as in translocations involving three or more chromosomes and in those involving the Y, an additional test was run by crossing the F_2 phenotypically normal males to stock multiple marker females to recheck linkages.

Previous work on X-ray effects with Drosophila has been performed using comparatively slow X-ray treatment. Large total doses have been given but they have been administered at a rate ranging from 50 to 300 roentgens (r) per minute. The most frequently used rate has been in the neighborhood of 100 r per minute, and at this rate it would take 20 minutes to administer a total dose of 2000 r. If cross-linkages are formed at once, it is possible that during this time some chromosome breaks would not have the opportunity to undergo cross-union due to restitution of the broken chromosome before a second break becomes available. Unless at least two chromosomes cross-join so as to establish a translocation, the breaks would not be detected by our present methods of analysis. The possibility of restitution of the original sequence might not be so great if the total dose were administered in a comparatively short period of time. The cellular mechanisms protecting against the radicals which produce indirect effects also increase the desirability of information concerning the effects of fast irradiation. The X-ray doses in some of these experiments were administered at the rate between 1800 and 1900 r per minute, while others were given at 100 r per minute.

RESULTS

The experimental results are given in table 1 and figures 1, 2, 3 and 4. In these last two figures, the crosslines indicate twice the standard error for the particular test as given in table 1. In some of these tests the total dose deviated 50 to 100 r units from the 2000 r desired. In these cases the 2000 r rate was estimated by interpolation, using the slope of BAKER's (1949) curve for cold slow irradiation. The interpolation is given in table 1, and these corrected figures (at the 2000 r level) are used in figures 3 and 4. These tests provide information on the relation of radiation damage, here measured as the frequency of translocations induced, to several variables: the rate of irradiation, temperature during irradiation, presence and concentration of chemically active metabolites. These gases, oxygen, carbon monoxide, carbon dioxide, and their mixtures, were present as atmosphere around the Drosophila during irradiation, with nitrogen used as the metabolically inert gas. The pre- and post-

TABLE 1

Rearrangements produced under different conditions.

Exp. Code No.*	Source	Gaseous environment of flies during irradiation	Temp. during irradiation (± 2 °C)	Total dosage of X-ray (r-units)	X-ray dosage rate per minute (r-units)	No. of sperm tested	Sperm with translocations	% sperm showing translocations at	
								Indicated total dosage	2000 r**
1	Baker (1949)	Air	2	998	100	1130	53	4.7 ± 0.6	
2	" "	"	"	1996	"	1021	147	14.4 ± 1.1	14.5
3	" "	"	"	2986	"	853	258	30.2 ± 1.6	
4	" "	"	28	1009	"	550	28	5.1 ± 0.9	
5	" "	"	"	2019	"	516	58	11.2 ± 1.4	11.1
6	" "	"	"	3025	"	477	104	21.8 ± 1.9	
7	(Current results)	"	5	1800	90	454	55	12.1 ± 1.6	14.1
8	" "	"	23	1900	"	455	37	8.1 ± 1.3	8.8
9	" "	"	0	500	1818	569	14	2.6 ± 0.7	
10	" "	"	3	1000	"	858	49	5.8 ± 0.8	
11	" "	"	0	2000	"	703	121	17.2 ± 1.4	17.2
12	" "	"	23	2000	"	598	83	13.9 ± 1.4	13.9
13	Baker, Edington (1952)	100% N_2	6	2000	100	512	19	3.7 ± 0.8	3.7
14	" " "	95% N_2 + 5% O_2	"	2000	"	525	52	9.9 ± 1.3	9.9
15	" " "	79% N_2 + 21% O_2	"	2000	"	616	99	16.1 ± 1.5	16.1
16	(Current results)	96% N_2 + 4% O_2	0	1976	104	496	25	5.0 ± 1.0	5.2
17	" "	" "	23	1960	98	434	28	6.5 ± 1.2	6.8
18	" "	" "	0	500	1818	530	11	2.1 ± 0.6	
19	" "	" "	2	1000	"	760	40	5.3 ± 0.8	

*The code number serves to locate the corresponding bar graph in figures 2 and 3.

**Where the total dosage in a given experiment was not 2000 r, the percent of sperm showing translocations was interpolated to 2000 r using the slope of Baker's curves at slow dose rate and at the corresponding temperature from figure 1. Such a correction rarely exceeded one percent.

TABLE 1 (*continued*)

Exp. Code No.*	Source	Gaseous environment of flies during irradiation	Temp. during irradiation (± 2°C)	Total dosage of X-ray (r-units)	X-ray dosage rate per minute (r-units)	No. of sperm tested	Sperm with translocations	% sperm showing translocations at	
								Indicated total dosage	2000 r**
20	(Current results)	96% N_2 + 4% O_2	2	2000	1818	505	71	14.1 ± 1.6	14.1
21	" "	" "	25	2054	1867	569	69	12.1 ± 1.4	11.7
22	Baker, Edington (1952)	100% O_2	6	2000	100	494	85	17.2 ± 1.7	17.2
23	(Current results)	99.5% O_2	3	1957	103	430	69	16.0 ± 1.7	15.3
24	" "	"	25	1960	98	509	63	12.4 ± 1.5	12.7
25	" "	"	0	2054	1867	505	97	19.2 ± 1.7	18.4
26	" "	"	25	2054	1867	517	102	19.7 ± 1.8	18.9
27	" "	80% CO_2 + 20% O_2	2	1890	90	508	94	18.5 ± 1.8	19.9
28	" "	" "	24	1900	95	503	73	14.5 ± 1.6	15.5
29	" "	" "	14	2054	1867	503	84	16.7 ± 1.6	16.1
30	" "	" "	25	2054	1867	303	57	18.8 ± 2.1	18.3
31	" "	80% CO + 20% O_2	5	2050	102	1048	196	18.7 ± 1.4	18.1
32	" "	" "	25	1932	92	524	89	17.0 ± 1.7	17.5
33	" "	" "	0	2054	1867	1099	216	19.6 ± 1.2	19.0
34	" "	" "	25	2054	1867	485	60	12.4 ± 1.5	11.9
35	" "	40% CO + 40% CO_2 + 20% O_2	5	2018	97	521	68	13.0 ± 1.5	12.8
36	" "	40% CO + 40% CO_2 + 20% O_2	28	1890	90	523	64	12.2 ± 1.5	13.1
37	" "	60% CO + 20% CO_2 + 20% O_2	23	2000	1818	504	45	8.9 ± 1.3	8.9
38	" "	95% CO + 5% O_2	3	2054	1867	575	101	17.6 ± 1.6	17.0
39	" "	" "	25	2054	1867	1033	185	17.9 ± 1.2	17.4
40	" "	47.5% CO + 47.5% CO_2 + 5% O_2	2	2000	1818	519	79	15.2 ± 1.6	15.2
41	" "	75% CO + 20% CO_2 + 5% O_2	23	2000	1818	664	77	11.6 ± 1.2	11.6

treatment in the gas at the temperature of the test insured, so far as possible, that the gases dissolved in the body fluids of the flies were at equilibrium with the atmosphere of gases surrounding them. The testing procedure used has provided a control to measure the magnitude of the spontaneous translocation rate. With about 10 F_1 progeny from each male tested, it was possible to determine if there were any spontaneous translocations present in the individually tested P_1 males. One cluster was found in which all F_1 males had the same translocation among 2367 P_1 males tested in all the experiments. This is a rate of 4.2×10^{-4}, but, of course, the rate is so low and the test so small that it is subject to a large error.

Most experimental tests were made using 2000 r because of the nature of the material as established by BAKER (1949) who tested *Drosophila virilis* at 1000, 2000 and 3000 r. Some of his material is included, as well as additional material from similar tests on the effect of oxygen (BAKER and EDINGTON 1952). These experiments were carried out using 500, 1000 and 2000 r to establish dosage rate curves, but did not go above 2000 r, because the frequency of complex translocations with more than two chromosomes involved became too high. In table 1 and figures 1–4, each sperm with a translocation (or translocations) is counted as one case. Table 2 gives the data so that two separable translocations are calculated as two cases. The experiments usually involved about 500 F_1 males which were tested for translocations, so the sampling error is larger than desirable.

Temperature effect

In these tests, more translocations were produced when the flies were irradiated at the lower temperature, 0–5°C, than at the higher temperature. This is in agreement with the results of MICKEY (1938) and BAKER (1949). The paired tests (differing only in temperature during irradiation) which did give a higher rate at the warm temperature involve small differences, while the majority of the experiments, including those with large differences between parallel experiments, show a higher rate of rearrangements in the cold. There are twelve pairs of tests which are otherwise comparable but differ in temperature in our 2000 r experiments. In eight sets, irradiation in the cold produced higher translocation rates and in one set the difference is statistically significant. None of the four sets shows significant difference when the rate of translocations is higher in the warm than the cold test. If we disregard other experimental conditions and contrast the translocation rate for the sum of the twelve cold tests with that of the twelve warm tests, the translocation rate is significantly higher at the lower temperature.

Rate effect

One interesting finding was that the rate of irradiation influenced the frequency of translocations produced. The best test to demonstrate the difference between the " fast rate " (over 1800 r per minute) and the " slow rate " (100 r per minute) is in material treated in nitrogen-oxygen mixtures, with only

TABLE 2

Translocation type and chromosome involved.

Experiment	Number tested	Types of translocations					Chromosomes involved					
		T_2	T_3	T_{2+2}	T_4	T_{2+3}	Y	2	3	4	5	
18	530	10	1				4	6	5	2	6	
9	569	14					4	2	5	10	7	
19	760	38		2			8	22	19	17	18	
10	858	49					11	22	23	19	23	
Total, lower dose	2717	111	1	2			27	52	52	48	54	4.20%
20	505	69	2				23	32	36	27	26	
21	569	67	1	1			11	31	29	38	30	
16	496	24	1				6	11	14	13	7	
17	434	28					10	10	8	14	15	
Total, N_2	2004	188	4	1			50	84	87	92	78	9.63%
11	703	109	10	2			33	56	56	54	53	
12	598	75	7	1			19	43	36	37	39	
7	454	53	2				7	27	25	21	32	
8	455	35	2				8	18	16	15	19	
Total, air	2210	272	21	3			67	144	133	127	143	13.39%
25	505	92	5				28	43	46	49	34	
26	517	93	5	4			28	50	51	36	44	
23	430	65	3	1			15	26	36	30	25	
24	509	60	3				20	26	33	20	30	
Total, O_2	1961	310	16	5			91	145	166	135	133	16.88%
29	503	77	5	2			24	41	34	35	39	
30	303	55	2				11	24	25	30	27	
27	508	88	3	3			27	35	41	39	49	
28	503	68	3	2			16	28	40	33	32	
Total 80 CO_2 + 20 O_2	1817	288	13	7			78	128	140	137	147	16.95%
31	1048	186	6	3	1		49	107	83	86	75	
32	524	84	4	1			17	39	39	39	48	
33	1099	203	11	2			61	115	86	83	98	
34	485	53	5	2			15	32	23	27	25	
38	575	96	3	1	1		25	49	42	37	54	
39	1033	175	7	1	2		50	95	76	82	78	
Total CO + O_2	4764	797	36	10	4		217	437	349	354	378	17.78%
35	521	65	2	1			29	29	25	24	31	
36	523	62	2				21	33	32	26	18	
37	504	43	2				7	25	15	24	21	
40	519	74	4			1	26	30	39	33	34	
41	664	68	8	1			18	35	36	39	34	
Total CO + CO_2 + O_2	2731	312	18	2		1	101	152	147	146	138	12.19%
All 2000 r	15487	2167	108	28	4	1						
Grand total	18204	2278	109	30	4	1	631 12.7	1142 23.0	1074 21.7	1039 21.0	1071 21.6	= 4957 %

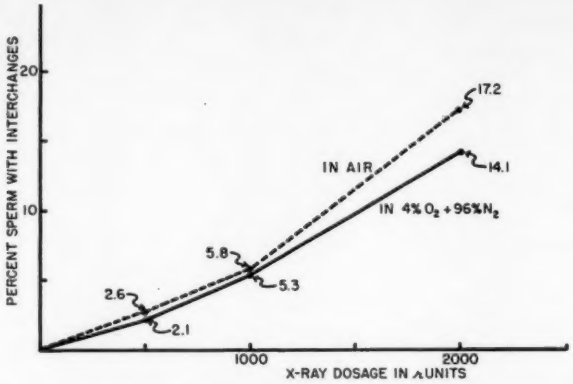

FIGURE 1.—The relationships between dosage and oxygen tension at rate 1867 r per minute.

oxygen of importance as gas environment in the indirect effects (fig. 3). The better data to demonstrate the greater effectiveness of the fast rate of irradiation is the material treated in 4% oxygen plus 96% nitrogen, where the oxygen effect is near the minimum. In these tests the frequency of translocations is two to three times as great if the irradiation is fast. When carbon monoxide

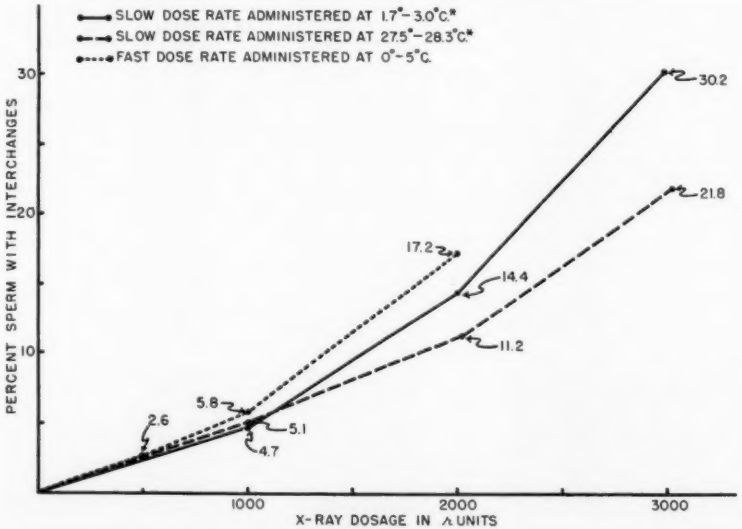

FIGURE 2.—The relationships between dosage, dose rate and temperature in the production of translocations in sperm by X-rays. The fast dose rate in all cases was administered at 1867 r per minute; the slow dose rate at approximately 100 r per minute.

* Slow dose rate curves are from BAKER 1949.

or carbon dioxide is present in addition to oxygen, the indirect effects produce more complex interactions, which may mask the rate effect. Figure 2 shows that the curve for the fast irradiation in the cold is similar to but has a somewhat steeper slope than those for slow irradiation at the two temperatures used by BAKER (1949). Figure 1 compares the effects of irradiating in air with the effect of irradiating in 4% oxygen plus 96% nitrogen. The higher concentration of oxygen increases the frequency of translocations at the doses of 500, 1000 and 2000 r units. The fast irradiation causes as much genetic damage with only 4% oxygen as slow irradiation causes in air (figs. 1 and 2). There are six paired tests in which Drosophila males were X-rayed 2000 r in nitrogen-oxygen mixtures. The translocation rate was higher in the progeny of Drosophila irradiated rapidly in each test. The difference is statistically significant in 4 percent oxygen or if the average rate for the six fast treatments is compared to that of the six slow treatments.

Oxygen effect

The effect of oxygen is obvious from figures 1 and 3, which include our results together with similar tests with *Drosophila virilis* by BAKER and EDINGTON (1952). Comparisons between the data from the other workers and

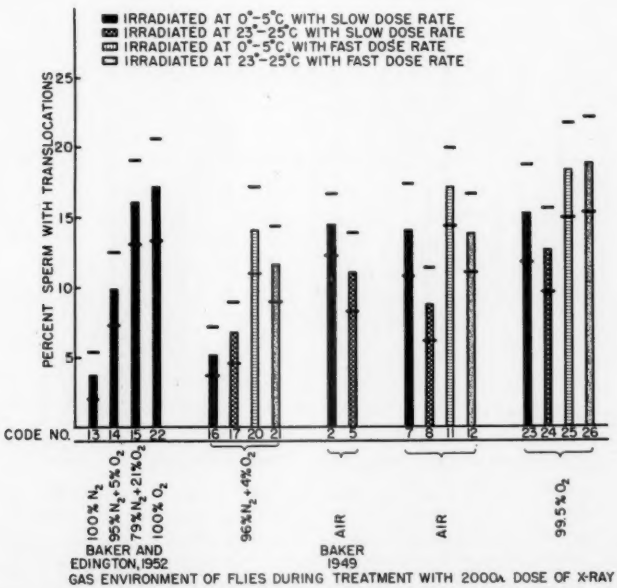
IRRADIATED AT 0°–5°C WITH SLOW DOSE RATE
IRRADIATED AT 23°–25°C WITH SLOW DOSE RATE
IRRADIATED AT 0°–5°C WITH FAST DOSE RATE
IRRADIATED AT 23°–25°C WITH FAST DOSE RATE
PERCENT SPERM WITH TRANSLOCATIONS
25
20
15
10
5
CODE NO. 13 14 15 22 16 17 20 21 2 5 7 8 11 12 23 24 25 26
100% N2
95% N2+5% O2
79% N2+21% O2
100% O2
96% N2+4% O2
AIR
AIR
99.5% O2
BAKER AND EDINGTON, 1952
BAKER 1949
GAS ENVIRONMENT OF FLIES DURING TREATMENT WITH 2000A DOSE OF X-RAY

FIGURE 3.—The effect of oxygen, temperature and dose rate on the production of translocations in sperm by 2000 r doses of X-rays. The slow dose rate was approximately 100 r per minute; the fast dose rate approximately 1800 r per minute. Each bar graph is keyed to the data in table 1 by the code number at the base of the graph.

these experiments show good agreement for similar tests. In both slow and fast irradiation these data show that, as the concentration of oxygen is increased during irradiation, the amount of genetic damage increases also. This is true in the series at 0–5°C and that at 23–25°C.

Other gases

The effects of carbon monoxide and of carbon dioxide were investigated in combinations with oxygen. Comparing results obtained in air with those in a mixture of 80% CO_2 plus 20% O_2, the radiation damage is distinctly higher with slow irradiation in the latter. However, the damage is not increased in fast irradiation at 14°C (number 29 on fig. 4), although it seems to be increased in the fast warm test. These findings agree with those of KING and SCHNEIDERMAN (1952) that CO_2 increases radiation damage in Tradescantia. On the other hand air compared with 80% CO plus 20% O_2 shows that there is a large increase in damage in the cold tests, both for slow and fast irradia-

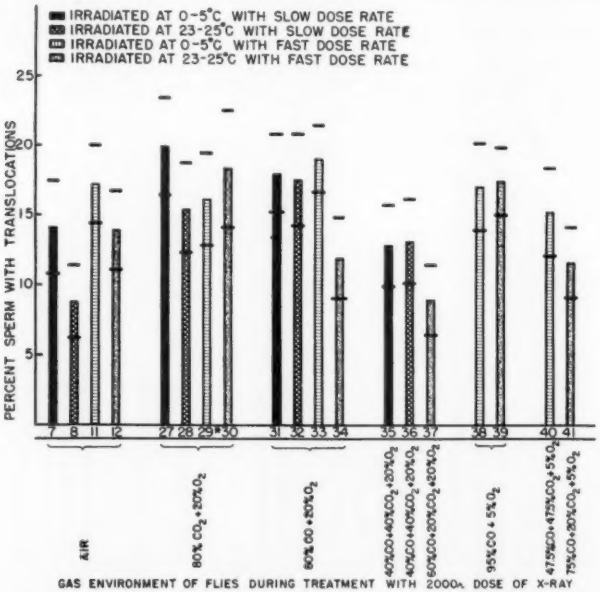
IRRADIATED AT 0-5°C WITH SLOW DOSE RATE
IRRADIATED AT 23-25°C WITH SLOW DOSE RATE
IRRADIATED AT 0-5°C WITH FAST DOSE RATE
IRRADIATED AT 23-25°C WITH FAST DOSE RATE
PERCENT SPERM WITH TRANSLOCATIONS
25
20
15
10
5
7 8 11 12 27 28 29 *30 31 32 33 34 35 36 37 38 39 40 41
AIR
80% CO2 + 20% O2
80% CO + 20% O2
40% CO + 40% CO2 + 20% O2
40% CO + 40% CO2 + 20% O2
60% CO + 20% CO2 + 20% O2
95% CO + 5% O2
47.5% CO + 47.5% CO2 + 5% O2
75% CO + 20% CO2 + 5% O2
GAS ENVIRONMENT OF FLIES DURING TREATMENT WITH 2000R DOSE OF X-RAY

FIGURE 4.—The related effects of carbon dioxide, carbon monoxide, oxygen, temperature and dose rate on the production of translocations in sperm by 2000 r doses of X-ray. The slow dose rate was approximately 100 r per minute; the fast dose rate approximately 1800 r per minute. Each bar graph is keyed to the data in table 1 by the code number at the base of the graph. The results in air, presented at the left, are included for comparison.

* No data for fast irradiation in 80% CO_2 + 20% O_2 at 0°C was available. The data used for construction of this graph was obtained at 14°C under these conditions of gas and dose rate.

tion. There is a large increase in damage in the warm slow test, which is comparable to the results of KING, SCHNEIDERMAN and SAX (1952). On the other hand the warm fast irradiation test shows less damage than obtained in air (number 34 on fig. 4). This apparent reduction is not due to a sampling error, since another test gave 41 translocations in 502 F_1's tested with 2054 r, or a corrected figure of 7.8% translocations. This result was obtained in an early experiment, in which we made carbon monoxide mixed with carbon dioxide, and then removed the latter by bubbling through a NaOH solution. Although our results probably are reliable, the fact that the mixture of $CO + CO_2 + O_2$ gives a low rate of translocations (see 37) caused us to disregard this experiment, in order to remove any possibility that the contaminating effect of appreciable amounts of CO_2 had lowered the value.

In the experiments with 95% CO plus 5% O_2, the fast cold treatment shows no difference from air, but the fast warm treatment (number 39 on fig. 4) may have produced a greater effect than the comparable air experiment, and is certainly significantly above the comparable 80% CO + 20% O_2 test. Every experiment with a mixture of $CO_2 + CO + O_2$ gave values for radiation damage below those obtained in other comparable tests having $CO + O_2$ or $CO_2 + O_2$ atmospheres. Agents which increase radiation damage are not necessarily additive in their effects.

Location of breaks

There is accurate information on the relative frequency of breakage in a particular chromosome involved in these translocations. Table 2 shows that the Y chromosome is involved in 12.7% of the translocations, chromosome 2 in 23.0%, chromosome 3 in 21.7%, chromosome 4 in 21.0% and chromosome 5 in 21.6%. Stated another way, translocations involving the Y are about six-tenths (0.583) as frequent as those involving an autosome. BAKER (1949) obtained similar results except that a smaller percent of translocations involved the Y. In his sample the Y was involved about four tenths (0.422) as often as the average for the autosomal frequency or 9.55% of 1749 chromosomes involved in his tests. The differences may represent differences in the *Drosophila virilis* stock tested or from sampling. In *Drosophila virilis* the Y chromosome is heterochromatic and as long or longer than the autosomal rods even though the latter have a large heterochromatic segment, one-third to one-half the chromosome (HEITZ 1935). The translocations recovered in these experiments less frequently involve an exchange with a break in the heterochromatin. Some part of the lower frequency of Y chromosome translocations is due to our failure to detect translocations with very viable aneuploid classes. The relative frequency of interchanges involving autosomes is in excellent agreement with expectation; for example, the second chromosome is slightly longer than the others in the salivary gland nuclei and is more frequently involved than any other autosome. A sample of 102 translocations between the Y and one of the major autosomes was analyzed cytologically by GEORGE VLAHAKIS (unpublished). The 25 or 26 breaks in each autosome appeared

to be distributed at random along the chromosome. This small sample would not show the restricted amount of local bunching of breaks demonstrated in the large number of breaks analyzed by KAUFMANN (1946) in *Drosophila melanogaster*.

DISCUSSION

CATCHESIDE (1948) and MULLER (1940, 1954) have reviewed the literature on the genetic effects of radiations. The results presented in table 1 provide certain additional information on chromosome breakage, although only the frequency of sperm with translocations which are viable and fertile is measured rather than the frequency of breaks. There is no sure way to separate the chromosome breaks produced directly by X-rays from those produced secondarily by the chemical mutagens which result from the ionization of water and other components of the biological system outside the chromosomes, or from those produced by the combined direct and indirect effects.

The overwhelming body of evidence from Drosophila, as illustrated by the early work of DEMPSTER (1940), demonstrated that intermittent doses of X-rays produced as many translocations as continuous irradiation. MULLER (1940) gave evidence which agreed with that of DEMPSTER and which showed in addition that the frequency of translocations for the same total dose was as great when the intensity was 0.05 r per minute as when it was 250 r per minute. Consequently it was concluded that breaks accumulate in the sperm and that rearrangements occur after fertilization. There was no evidence for healing through time during sperm storage. These and other tests reviewed by CATCHESIDE (1948) and MULLER (1954) indicate that the number of symmetrical rearrangements depends on the total amount of X-radiation irrespective of the intensity or fractionation of the dose.

In Tradescantia most tests indicate both intensity and fractionation effects. As an example, SAX and LUIPPOLD (1952) presented information on the frequency of two-hit chromosome aberrations which demonstrated an effect of both intensity and fractional dosage. DE SERRES and GILES (1953) have recently presented additional evidence on this effect with X-rays. However, GILES, DE SERRES and BEATTY (1953) showed that there is no effect due to radiation dose fractionation with fast neutrons. The frequency of chromosomal aberrations is directly proportional to the amount of radiation from neutrons. Our experiments, especially those in figure 3, present evidence that there is an intensity effect when the dosage was given at 2000 r in sixty-six seconds as compared to 2000 r in twenty minutes. We cannot ascribe the intensity effect to differences in wave lengths of X-rays used in different tests, although KIRBY-SMITH and DANIELS (1953) have demonstrated a difference in the effectiveness of X-rays and gamma rays from cobalt^{60} in producing chromosome breakage in Tradescantia. In numerous tests by other workers, using both Tradescantia and Drosophila, no such wave length dependence has been demonstrated within the range of wave lengths from X-rays used in our experiments.

We can make a rough comparison of the effects of different physiological conditions of the sperm during irradiation using results from 2000 r units X-radiation (table 2). Although it introduces some bias because of difference in sample size and other factors, we can lump all cultures treated in certain gas combination and compare the percentages of translocations. In 96% nitrogen plus 4% oxygen, 9.63% of 2004 fertile F_1 cultures had translocations present; in air 13.39% of 2210 cultures; in 99.5% oxygen 16.88% of 1961 cultures; in carbon dioxide–oxygen mixtures 16.95% of 1817 cultures; in carbon monoxide–oxygen mixtures 17.78% of 4764 cultures; in carbon monoxide–carbon dioxide–oxygen mixtures only 12.19% of 2731 cultures. Even in these average effects, the low oxygen atmosphere (96% N_2 + 4% O_2) results in least damage, air is lower than the next three combinations of gases, while $CO_2 + CO + O_2$ results in slightly less damage than air. With these size samples, a difference of 2.0% (perhaps less) is statistically significant.

In our experiments radiation damage was usually greater at 3°C than at 25°C. The relations between temperature at the time of irradiation and the oxygen effect are quite interesting. The higher temperature, 25°C, is about optimal for *Drosophila virilis*, while at 3°C the flies are immobilized and their metabolic processes are slowed down, although they are able to survive for considerable periods of time. Oxygen is nearly 1.6 times as soluble in water at 3°C as at 25°C. Although oxygen in the atmosphere may be at equilibrium with that dissolved in the body fluids of the fly at 3°C, the active metabolism at 25°C will tend to reduce the concentration of dissolved oxygen even below two-thirds of the amount present at the lower temperature because of the difference in solubilities. This difference in oxygen present in the cells during irradiation, as well as the greater activity of other biological protective agents at 25°C, might account for much of the temperature differential. Chromosome breakage by the mechanism demonstrated by CONGER and FAIRCHILD (1952) may also contribute, particularly as irradiation may partially disrupt the normal protective mechanisms of the cell. Their tests show that oxygen may act as a chemical mutagen, leading to chromosome breakage. This action is independent of radiations although this effect and combined effects are synergistic with radiations and increase radiation damage.

FABERGÉ (1950a, b) showed that at temperatures above freezing but not at − 192°C the concentration of oxygen affects the amount of damage from X-radiation. NYBOM, LUNDQVIST, GUSTAFSSON and EHRENBERG (1953) published extensive tests of the effect of temperature differences on growth, fertility, mutation rate and chromosome abnormality rate in barley. They tested different doses of X-rays at temperatures of + 20°C and − 190°C. They conclude that X-ray damage is less at low temperature using any of the measures of injury they tested. The low temperature reduces both physiological and genetic damage by eliminating part or most of the indirect effect. This oxygen effect was demonstrated by THODAY and READ (1947), GILES and RILEY (1949, 1950), HAYDEN and SMITH (1949), RILEY, GILES and BEATTY (1952) and numerous others on chromosome breaks. There are two hy-

potheses to explain this effect. The first presumes that oxygen forms mutagenic compounds in tissues during irradiation which increases the amount of damage including the number of breaks. The second assumes that oxygen during irradiation interferes with the restitution process so that more interchanges can occur but that it does not increase the number of breaks. In so far as this is an alternative to the first hypothesis (of course both things may happen) it must assume that breakage is due to the direct action of the X-radiation and not to free radicals and peroxides formed by the ionizations of water and organic materials, for oxygen certainly contributes to these. Most investigators, including MULLER (1954) who reviews the evidence, assume that oxygen contributes chemical mutagens which increase the number of chromosome breaks. However SCHWARTZ (1952) and BAKER and VON HALLE (1953) have advocated that the oxygen prevents restitution and so increases the opportunity for crosslinkages to be formed. Their arguments are not convincing because they cannot justify their basic premise. Before they can restrict the effect of oxygen to a role in reunion, they must assume that although oxygen can break chromosomes and produce rearrangements without irradiation (CONGER and FAIRCHILD 1952), it cannot cause breakage if it is present when the material is irradiated nor can it form active radicals which break chromosomes under these circumstances. The evidences bearing on the role of the direct and indirect effects of radiations are discussed elsewhere (STONE et al. 1954).

We know that random reunion of the segments of broken chromosomes does not occur. As CATCHESIDE (1948) pointed out, several earlier investigations using Drosophila showed an excess of inversions as compared to translocations. In these latter, there is an excess of two-by-two rearrangements over those with all four chromosomes involved together. We have 34 cases where breaks in four chromosomes were present: 30 of these were cases with two separate translocations and only 4 had all four chromosomes linked, table 2. These results agree with those of BAKER (1949) for material irradiated 2000 r. Only one case was found with all five chromosomes involved; it was two translocations, one involving two and the other three chromosomes. The tendency to form two chromosome exchanges more frequently than the 1 : 2 ratio expected by chance for two-by-two to all four chromosomes might be explained as a result of a proximity limitation. Drosophila resembles Tradescantia in this respect for in the latter LEA and CATCHESIDE (1942) showed that crossunions occur between broken ends lying close together in the nucleus rather than at random. A discussion of the implications of this and other related material is given elsewhere (STONE et al. 1954).

Radiation damage varies with variations in the physiological state of the organism. BONNIER and LÜNING (1950) and LÜNING (1952a, b) showed that the probability that X-radiation would produce chromosomal abnormalities varied with the stage in the chromosome cycle in *Drosophila melanogaster* just as in other organisms. The effects of oxygen, carbon dioxide, and carbon monoxide in modifying X-ray effects are through the physiological systems

rather than through modification of the ionizations produced by irradiation. DALE (1952) states that the indirect effects of irradiation are very pH dependent. SIZOVA (1936) published a note on the effect of X-rays combined with carbon dioxide or ammonia using *Crepis capillaris* as test organism. Each agent increased chromosomal aberration frequency in combination with X-rays. Later work with different organisms has not always been in agreement. ZIRKLE (1940) showed that lowering the pH with carbon dioxide increased radiation damage.

This effect of CO_2 was consistent at different concentrations when 4.5-hour *Drosophila melanogaster* eggs were irradiated. Eggs irradiated only 2 hours after being laid varied in sensitivity with concentration of CO_2. ZIRKLE (1941) found a similar variation with concentration of CO_2 and of NH_3 in tests using reduction in percentage of cell division in germinating fern spores. In this last paper ZIRKLE showed that there was a difference in response with two intensities of X-rays. ZIRKLE concludes that change in the physiological conditions of the organism influences the radiosensitivity of several different systems which are damaged by irradiation. SCHNEIDERMAN and KING (1953) tested the effect of CO_2 on chromosome damage by X-rays in Tradescantia. In pure CO_2 the number of abnormalities produced by X-rays was equivalent to that in a vacuum. If CO_2 is added to one atmosphere of air, there is a rapid increase in percent of rearrangements above that in air to about 1.8 times at 0.1 atmosphere CO_2. Thereafter the increase in damage with increase in CO_2 is less rapid but consistent, unlike the variations found by ZIRKLE. If the CO_2 was added immediately after irradiation, there was no increase in rearrangement rate. They conclude that the CO_2 influences the indirect effects of irradiation which involve oxygen. Our own results with carbon dioxide show decided variations with different physiological conditions. We have no test of effectiveness in relation to concentration. Some of the variations in radiation damage in the mixtures of CO_2 with O_2 and CO might be due to concentration effects such as ZIRKLE reported.

The relation of the cytochrome system to radiation damage was investigated with carbon monoxide mixtures during irradiation. The effect of this enzyme poison is quickly reversed in Drosophila when the organisms are returned to air so that oxygen must reactivate the cytochrome system as suggested by KING, SCHNEIDERMAN and SAX (1952) from their work in Tradescantia. This is borne out in our material where X-ray induced translocations are more frequent in 95% CO + 5% O_2 (39) than in 80% CO + 20% O_2 (34) at the normal temperature for physiological activity in Drosophila. Test 33 indicates that oxygen does not restore the protective capacity of the cytochrome system at 0°C. When test 32 is compared to 34, we find that some types of injury must require slower irradiation and require time for cumulative action to effect breaks.

Carbon dioxide reduces the radiation damage ordinarily occurring in carbon monoxide–oxygen mixtures in most combinations of temperature and gas mixtures. In fact the frequency of translocations is reduced to the lowest values

for 2000 r in 66 seconds at 25°C, the temperature where most enzyme systems will be active.

SUMMARY

Investigations were made to determine the extent of X-radiation damage due to indirect effects by varying the physiological conditions during irradiation of the test organism, *Drosophila virilis*. Genetic damage was measured as recoverable translocations, which involve breakage and recombination of parts of two or more chromosomes. The effects of temperature, oxygen, carbon monoxide, and carbon dioxide in various combinations were tested, using two rates of irradiation, fast at approximately 2000 r per minute and slow at 100 r per minute.

One spontaneous translocation was found in 2367 tests where such translocations could be detected. Therefore the spontaneous rate is too low to modify any data appreciably.

In air and other oxygen-nitrogen mixtures, where we can regard nitrogen as inert and oxygen as an active agent in the indirect effects producing radiation damage, there is a direct relation between the amount of oxygen in the gas and the genetic changes. As the amount of oxygen increases, the damage to the chromosomes increases. The greater damage at 0°C compared to 25°C may be attributed to the greater solubility (1.6×) of oxygen in water at the lower temperature, together with a greater saturation of the oxygen receptors with increased concentration of oxygen.

In tests using oxygen–nitrogen mixtures, the amount of induced genetic change is much greater with fast irradiation than with slow. If we lump all paired tests into fast versus slow irradiation, we have over five thousand tested cultures in each. In eight of the ten pairs, damage was greater at the fast rate. The average was $15.95 \pm 0.5\%$ for fast versus $13.39 \pm 0.5\%$ for slow irradiation, with the difference $2.56 \pm 0.7\%$ for all experiments. As there is no evidence for healing in the sperm, this must be due to the rapid production of such a high concentration of active radicals in the water and their reactive derivatives that the natural protective systems, which reduce the damage due to these chemical effects, are unable to protect the chromosomes.

If nitrogen is replaced by carbon dioxide, the radiation damage is increased, particularly with slow irradiation. The increase in carbon dioxide concentration may lower the pH, disrupt the regular oxidation-reduction systems, and increase the concentration and stability of OH, O_2H and H_2O_2; each would contribute to radiation damage. Carbon monoxide, which ties up the cytochrome and so interferes with the destruction of oxidative radicals, increases radiation damage in slow doses and fast doses at 2°C, but not fast doses at 25°C, another illustration of the complexity of the protecting systems. The fact that both carbon dioxide and carbon monoxide, which can influence only the indirect chemical effects of radiation, increase damage with slow irradiation to equal that with fast irradiation indicates that the greater damage of the latter in oxygen–nitrogen mixtures is due to the greater concentration of

active radicals, leading to chemical damage to the genetic system. When all the tests are compared, the indirect effects of radiation account for more than 50 percent of all demonstrable genetic changes in *Drosophila virilis*.

Mixtures of CO, CO_2 and O_2 produce less radiation damage than either $CO + O_2$ or $CO_2 + O_2$, again indicating that the protective systems and interaction of active radicals are very complex problems and that these types of radiation damages are not simply cumulative.

The Y chromosome was involved about six-tenths as frequently as any one major autosome. The Y is heterochromatic and somewhat longer than any autosome in the ordinary metaphase. As from one-third to one-half the length of the autosomes is due to heterochromatin, our tests show a greater rearrangement frequency for the euchromatin.

Among those translocations with four chromosomes involved, the relative frequency of two-and-two translocations to all four chromosome translocations shows that reunion of broken chromosome ends is not at random in *Drosophila virilis*.

Protection against radiation damage by the cell seems to be due to the physiological mechanisms regularly involved in oxidative metabolism such as the —SH compounds and the cytochrome, catalase, and correlated systems.

ACKNOWLEDGMENT

Calibrations of the X-ray equipment and measurement of X-ray dosages were made by DR. IBEN BROWNING of the Biology Department and DRS. ROBERT J. SHALEK and PETER WOOTTON of the Physics Department of the M. D. Anderson Cancer Hospital at Houston, Texas. We wish to express our appreciation to them and to DR. R. LEE CLARK, JR., Director of the M. D. Anderson Cancer Hospital, for making the radiation equipment and laboratories available to us. DR. MARY L. ALEXANDER, MR. L. HERBERT BRUNEAU, and MR. PAUL MOORHEAD assisted us with some tests. This project was supported by the Atomic Energy Commission by contract AT-(40-1)-1323, and by the Rockefeller Foundation.

BIBLIOGRAPHY

AUERBACH, C., and J. M. ROBSON, 1946 Chemical production of mutations. Nature **157**: 302.

BAKER, W. K., 1949 The production of chromosome interchanges in *Drosophila virilis*. Genetics **34**: 167–193.

BAKER, W. K., and C. W. EDINGTON, 1952 The induction of translocations and recessive lethals in Drosophila under various oxygen concentrations. Genetics **37**: 665–677.

BAKER, W. K., and ELIZABETH S. VON HALLE, 1953 The basis of the oxygen effect on X-irradiated Drosophila sperm. Proc. Nat. Acad. Sci. **39**: 152–161.

BONNIER, G., and K. G. LÜNING, 1950 X-ray induced dominant lethals in *Drosophila melanogaster*. Hereditas **36**: 445–456.

CATCHESIDE, D. G., 1948 Genetic effects of radiations. Adv. in Genetics, vol. **2**: 271–358. Academic Press: New York.

CHINO, M., 1941 New mutants in *Drosophila virilis virilis*. Jap. J. Genet. **17**: 185–206.

CONGER, ALAN D., and LUCILE M. FAIRCHILD, 1952 Breakage of chromosomes by oxygen. Proc. Nat. Acad. Sci. **38:** 289–299.

DALE, W. M., 1952 Some aspects of the biochemical effects of ionizing radiations. Symposium on Radiobiology. John Wiley and Sons: New York, pp. 177–188.

DEMPSTER, E. R., 1940 Absence of a time factor in the production of translocations in Drosophila sperm by X-radiation. Amer. Nat. **75:** 184–187.

DE SERRES, F. J., and N. H. GILES, 1953 The effect of radiation dose fractionation on chromosome aberration frequencies in Tradescantia microspores. I. Studies with X-rays. Genetics **38:** 407–415.

FABERGÉ, A. C., 1950a Chromosome breakage by X-rays at low temperature and the radiodecomposition of water. Genetics **35:** 104–105.

1950b Relation between the action of cold and of nitrogen in decreasing the frequency of chromosome aberrations. Genetics **35:** 663.

GILES, N. H., F. J. DE SERRES and A. V. BEATTY, 1953 The effect of radiation dose fractionation on chromosome aberration frequencies in Tradescantia microspores. II. Studies with fast neutrons. Genetics **38:** 416–420.

GILES, N. H., JR., and H. P. RILEY, 1949 The effect of oxygen on the frequency of X-ray induced chromosomal rearrangements in Tradescantia microspores. Proc. Nat. Acad. Sci. **35:** 640–646.

1950 Studies on the mechanism of the oxygen effect on the radiosensitivity of Tradescantia chromosomes. Proc. Nat. Acad. Sci. **36:** 337–344.

HAAS, FELIX, E. DUDGEON, F. E. CLAYTON and W. S. STONE, 1952 Frequency of chromosomal rearrangements as related to rate of irradiation, temperature, and gases. Genetics **37:** 589–590.

HAYDEN, B., and L. SMITH, 1949 The relation of atmosphere to biological effects of X-rays. Genetics **34:** 26–43.

HEITZ, E., 1935 Chromosomenstruktur und gene. Zeit. ind. Abst. Ver. **70:** 402–447.

KAUFMANN, B. P., 1946 Organization of the chromosome. I. Break distribution and chromosome recombination in *Drosophila melanogaster*. J. Exp. Zool. **102:** 293–320.

KING, E. D., and H. A. SCHNEIDERMAN, 1952 The effects of carbon dioxide on the frequency of X-ray induced chromosome aberrations in Tradescantia. Proc. Nat. Acad. Sci. **38:** 809–812.

KING, E. D., H. A. SCHNEIDERMAN and K. SAX, 1952 The effects of carbon monoxide and oxygen on the frequency of X-ray induced chromosome aberrations in Tradescantia. Proc. Nat. Acad. Sci. **38:** 34–43.

KIRBY-SMITH, J. S., and D. S. DANIELS, 1953 The relative effects of X-rays, gamma rays, and beta rays on chromosomal breakage in Tradescantia. Genetics **38:** 375–388.

KOTVAL, J. P., and L. H. GRAY, 1947 Structural changes produced in microspores of Tradescantia by alpha-radiation. J. Genet. **48:** 135–154.

LEA, D. E., 1946 Actions of Radiations on Living Cells. Macmillan Co.: New York.

LEA, D. E., and D. G. CATCHESIDE, 1942 The mechanism of the induction of chromosome aberrations in Tradescantia. J. Genet. **44:** 216–245.

LÜNING, K. G., 1952a X-ray induced dominant lethals in different stages of spermatogenesis in Drosophila. Hereditas **38:** 91–107.

1952b X-ray induced chromosome breaks in *Drosophila melanogaster*. Hereditas **38:** 321–338.

MICKEY, G. H., 1938 Effect of temperature on the frequency of translocations produced by X-rays in Drosophila. Genetics **23:** 160.

MULLER, H. J., 1927 Artificial transmutation of the gene. Science **66:** 84.

1940 An analysis of the process of structural change in chromosomes of Drosophila. J. Genet. **40:** 1–66.

1954 The nature of the genetic effects produced by radiation. (and) The manner of production of mutations by radiation. Chapters 7 and 8 in Radiation Biology (Alexander Hollaender, editor). McGraw-Hill: New York.

NYBOM, N., U. LUNDQVIST, A. GUSTAFSSON and L. EHRENBERG, 1953 Biological effects of X-radiation at low temperatures. Hereditas **39:** 445–457.

RILEY, H. P., N. H. GILES, JR. and A. V. BEATTY, 1952 The effect of oxygen on the induction of chromatid aberrations in Tradescantia microspores by X-irradiation. Amer. J. Bot. **39:** 592–597.

SAX, K., and H. LUIPPOLD, 1952 The effect of fractional X-ray dosage on the frequency of chromosome aberrations. Heredity **6:** 127–131.

SCHNEIDERMAN, H. A., and E. D. KING, 1953 Further studies on the effects of carbon dioxide and oxygen on the frequency of X-ray induced chromosome aberrations in Tradescantia. Proc. Nat. Acad. Sci. **39:** 834–838.

SCHWARTZ, DREW, 1952 The effect of oxygen concentration on X-ray-induced chromosome breakage in maize. Proc. Nat. Acad. Sci. **38:** 490–494.

SIZOVA, M. A., 1936 Structural changes in chromosomes induced by irradiation of physiologically modified cells. Compt. Rend. U.R.S.S. **2:** 197–198.

STONE, WILSON S., FELIX HAAS, MARY L. ALEXANDER and FRANCES E. CLAYTON, 1954 Comments on the mechanism of action of radiations on living systems. University of Texas Bulletin, in press.

STONE, W. S., ORVILLE WYSS and FELIX HAAS, 1947 The production of mutations in *Staphylococcus aureus* by irradiation of the substrate. Proc. Nat. Acad. Sci. **33:** 59–66.

THODAY, J. M., and JOHN READ, 1947 Effect of oxygen on the frequency of chromosome aberrations produced by X-rays. Nature **160:** 608.

WYSS, ORVILLE, W. S. STONE and J. BENNETT CLARK, 1947 The production of mutations in *Staphylococcus aureus* by chemical treatment of the substrate. J. Bact. **54:** 767–772.

ZIRKLE, RAYMOND E., 1940 The influence of intracellular acidity of the radiosensitivity of various organisms. J. Cell. Comp. Physiol. **16:** 301–311.

1941 Combined influence of X-ray intensity and intracellular acidity on radiosensitivity. J. Cell. Comp. Physiol. **17:** 65–70.

GENETICS OF NATURAL POPULATIONS. XXII. A COMPARISON OF THE CONCEALED VARIABILITY IN DROSOPHILA PROSALTANS WITH THAT IN OTHER SPECIES[1]

THEODOSIUS DOBZHANSKY AND BORIS SPASSKY

Received December 2, 1953

DROSOPHILA PROSALTANS occupies a rather unique position among the species of Drosophila which have served as materials for studies on genetic population structure. For obvious technical reasons, these materials are usually chosen amongst species that are common, widespread, and occur in a variety of habitats. *D. melanogaster* is one of the best laboratory animals, and is also an "animal weed" which has reached a near-cosmopolitan distribution as a commensal in human households. *D. ananassae, D. virilis, D. simulans* and *D. immigrans* are also often associated with man, although they are not quite as widely distributed as *D. melanogaster. D. pseudoobscura, D. robusta* and *D. willistoni* are rarely commensals, but they are the commonest, or among the commonest, species of the genus in, respectively, western North America, eastern North America, and tropical America. In particular, *D. willistoni* is the most ubiquitous species in Brazil, and in many parts of that country is also the commonest. Now, *D. prosaltans* has a geographic distribution which resembles, in gross outline, that of *D. willistoni*; the former is known to occur from Cuba and central Mexico to southern Brazil and Paraguay, while the latter extends from southern Florida to La Plata in Argentine. But in contrast to *D. willistoni, D. prosaltans* is on the whole a rare species. It occurs chiefly in isolated pockets, and seldom builds dense populations even in these pockets (DOBZHANSKY and PAVAN 1950; PAVAN 1952). It is probable that *D. prosaltans* occupies some specialized ecological niches, although it is not known at present just what these niches are.

A comparison of the spontaneous mutation rates for lethals in the autosomes of *D. willistoni* and *D. prosaltans* has indicated that the latter species is only about half as mutable as the former (DOBZHANSKY, SPASSKY and SPASSKY 1953). Mutation rates are, however, variable also in different strains of the same species. More enlightening is a comparison of the magnitude of the stores of variabilities concealed in natural populations of these species. PAVAN et al. (1950) found high and fairly uniform frequencies of autosomal lethals and semilethals in populations of *D. willistoni* from various parts of Brazil. TOWNSEND (1952) studied geographically marginal populations of this species from Florida and Cuba, and found them to contain slightly fewer concealed lethals than occur in Brazil. By contrast, CAVALCANTI (1950) found fewer lethals in *D. prosaltans,* especially in the third chromosomes. His ma-

[1] The work reported in this article has been carried out under contract No. AT-(30-1)-1511, U. S. Atomic Energy Commission.

terial came chiefly from one locality in southern Brazil. The purpose of the present work is to extend the study made by CAVALCANTI. As will be shown below, we can confirm his findings on a larger material from several localities. The stores of variability carried in natural populations of *D. willistoni* and *D. prosaltans* are considerably different.

MATERIAL

Drosophila prosaltans is so rare in most localities in Brazil in which collections of Drosophila have been made that adequate population samples are hard to come by. DR. A. B. DA CUNHA sent us a good sample which he collected in 1952 at Barreiras, Bahia, Brazil. Between July 1 and 6, 1952, another good sample was collected by MISS SOPHIE DOBZHANSKY and the senior author near Cabo Maguary, on the Isle of Marajo, just south of the Equator. A fair collection was also made on July 18–23 of the same year at Fordlandia, on River Tapajos, state of Para. Some specimens have been obtained in the Territory of Amapa, on upper Rio Negro, near Belem, state of Para, near Lagoa Coelho in the state of Piaui, on upper Rio Doce in the state of Minas Geraes, and near Pirassununga, in the state of São Paulo. The material of CAVALCANTI (1950) was derived chiefly from the coast of São Paulo (Bertioga). The material on which the present work is based came, therefore, from different parts of Brazil, although some parts are represented by larger samples than others.

THE CROSSES

The techniques of detection of the variability concealed in the gene pool of a natural population of Drosophila have often been described. In the present work we have followed a procedure analogous to that used in the analysis of *Drosophila pseudoobscura* and *D. persimilis* (DOBZHANSKY and SPASSKY 1953, and earlier work). In the experiments with the second chromosomes, wild males or sons of wild females were crossed to females which carried the dominant gene Lobe in one of the second chromsomes; a single Lobe male from the F_1 was crossed to females which had the dominant mutants Plum Star Curly and an inversion in a second chromosome; in the next generation Plum Star Curly non-Lobe females and males were inbred, and counts were made in their offspring. In the experiments with the third chromosomes, wild males or sons of wild females were crossed to females homozygous for abdomen rotatum; a single F_1 male was crossed to females having the dominant Delta, the recessive abdomen rotatum, and an inversion in one of the third chromosomes. Delta females and males were selected in the next generation and inbred; counts were made in their offspring.

The inversions in the Plum Star Curly and Delta abdomen rotatum chromosomes were induced by X-ray treatments made especially for the purpose of the experiments here described. The inversions suppress most of the detectable crossing over in the chromosomes which carry them. The inversion in the second chromosome is cytologically pericentric, and includes slightly

TABLE 1

Percentages of wild-type flies in the control experiments.

Chromosome	Locality	Flies counted	Percent wild-type
Second	Barreiras	56,780	33.73
"	Marajo	16,630	34.16
"	São Paulo (Cavalcanti)	17,607	31.86
Third	Barreiras	47,784	37.71
"	Marajo	11,947	37.54
"	Miscellaneous	6,013	35.14
"	São Paulo (Cavalcanti)	2,138	42.89

more than half of the length of both arms of the chromosome. Among the 1545 flies of the original test, this inversion gave no crossing over between the genes *Pm, S* and *Cy,* and very few crossovers were observed in further work.[2] The inversion in the third chromosome is cytologically shorter—it includes about one-third of the chromosome. It permits about 0.15 percent of crossing over between Delta and abdomen rotatum (in 1229 flies). For further information concerning the experimental procedures see PAVAN et al. 1951, and DOBZHANSKY and SPASSKY 1953.

NORMAL VIABILITY AND DEVELOPMENT RATE IN HETEROZYGOTES

Control experiments are arranged by crossing flies which carry the same marking chromosome (the second or the third), but which contain wild chromosomes derived from different wild progenitors collected in the same locality. Wild-type flies obtained in the progeny of such crosses carry two chromosomes of a pair chosen at random from the population of a certain locality. Such wild-type flies are almost always heterozygotes, since the chromosomes derived from different individuals are, in cross-fertilizing species, usually unlike in gene contents. The viability of such flies is normal by definition (see PAVAN et al. 1951; DOBZHANSKY and SPASSKY 1953 and earlier work).

[2] CAVALCANTI (1950, table 1) records, however, up to 10 percent crossovers in his cultures. These could have been only phenotypic overlaps in the manifestation of the gene Curly. This does not invalidate his other conclusions, since in the calculations the supposed crossovers were added to the *Pm S Cy* class.

TABLE 2

Percentages of wild-type flies on different days of batching in the control experiments.

Counts	Second chromosome		Third chromosome	
	Flies counted	Percent wild	Flies counted	Percent wild
1	19,596	42.50	19,325	45.70
2	28,506	32.86	21,759	36.49
3	15,673	28.53	13,498	31.11
4+	9,688	27.57	5,942	27.36
Total	73,463	33.81	60,524	37.33

The results of the control experiments are summarized in tables 1 and 2. In the experiments with second chromosomes, the marking chromosome carried the three dominant genes Plum, Star, and Curly. Neverthless, the percentages of the wild-type flies obtained do not differ significantly from the ideal 33.33 (table 1). This means that the viability of the flies which carried the marking chromosome was approximately normal. In the experiments with third chromosomes, the dominant marker Delta reduces the viability of its carriers sufficiently to push the percentages of the wild-type class above 33.33. Since the control experiments for different localities were not made simultaneously, the data in table 1 are given separately for the localities. Except for the tests made by CAVALCANTI using a different food medium, the results of the experiments are reasonably uniform.

In all experiments, the flies hatching in the cultures were counted at intervals of 3 days. Table 2 shows the percentages of the wild-type class in the successive counts (the data are obtained by summing together the results in all cultures in which 3 or more counts have been made). It can be seen that the proportion of the wild-type class is highest in the first count and steadily declines thereafter. This means that the marking chromosomes with their mutants cause the flies carrying them to develop more slowly than do the normal wild-type flies.

LETHALS AND SEMILETHALS

The effects of homozygosis on the viability have been studied for 304 second and 284 third chromosomes. The results are summarized in tables 3 and 4 and in figure 1. The viability of the homozygotes is expressed in percentages of the normal viability. For this purpose, the frequency of the wild-type class observed in the cultures in which the homozygotes for a given wild chromosome are produced are divided by the frequency of the wild-type class in the corresponding control experiment (table 1), and multiplied by 100.

Tables 3 and 4 and figure 1 show the characteristic frequency distributions of the viabilities of the homozygotes for wild chromosomes. As in other species of Drosophila, these distributions are clearly bimodal. One peak lies just below the normal viability (the 90–100 percent class), and the other in the lethal class (0). The notch between the peaks is in the vicinity of 50 percent. This bears out the conventional definition of semilethals as genotypes which cause more than 50 percent, but less than 100 percent mortality of their carriers. For purposes of comparison, the most convenient figure is the frequency of lethal and semilethal chromosomes combined. From the data in tables 3 and 4, these frequencies are as follows:

Second chromosomes	Third chromosomes
$32.6 \pm 2.7\%$	$9.5 \pm 1.7\%$

The frequency of lethal and semilethal chromosomes is not quite uniform in the different localities studied. For the second chromosomes, it is relatively low at Barreiras and on Marajo Island, and high in the sample studied by CAVALCANTI and the sample from miscellaneous localities (table 3). A sta-

TABLE 3

Viability of homozygotes for second chromosomes, expressed in percent of the normal viability; the figures indicate numbers of chromosomes that fall in the different viability classes.

Locality	Lethal	0	10	20	30	40	50	60	70	80	90	100	110	120	130	140	150	Total
Barreiras	24	4	1	....	2	....	2	3	3	13	19	29	7	1	....	1		109
Marajo	17	2	1	2	1	....	2	2	3	10	27	13	2	....	....	....		82
Tapajos	2	....	1	....	....	....	....	2	2	6	4	1	....	....	....	....		18
Miscellaneous	9	1	....	....	1	....	....	....	1	1	3	4	....	1	....	....		21
São Paulo (Cavalcanti)	8	14	5	2	1	1	1	8	14	9	6	4	1	....	....	....		74
Total	60	21	8	4	5	1	5	15	23	39	59	51	10	2	....	1		304

TABLE 4

Viability of homozygotes for third chromosomes, expressed in percent of the normal viability; the figures indicate numbers of chromosomes that fall in the different viability classes.

Locality	Lethal	0	10	20	30	40	50	60	70	80	90	100	110	120	130	140	Total
Barreiras	7	1	....	2	....	....	2	1	1	17	38	32	3	1	....		105
Marajo	2	2	2	....	1	1	1	5	1	13	37	20	8	....	....		93
Tapajos	1	....	....	....	....	....	....	....	1	1	6	3	2	....	....		14
Miscellaneous	4	....	....	....	1	....	....	....	....	1	7	6	2	....	....		21
São Paulo (Cavalcanti)	....	....	....	....	1	2	1	1	5	8	8	15	5	4	1		51
Total	14	3	2	2	3	3	4	7	8	40	96	76	20	5	1		284

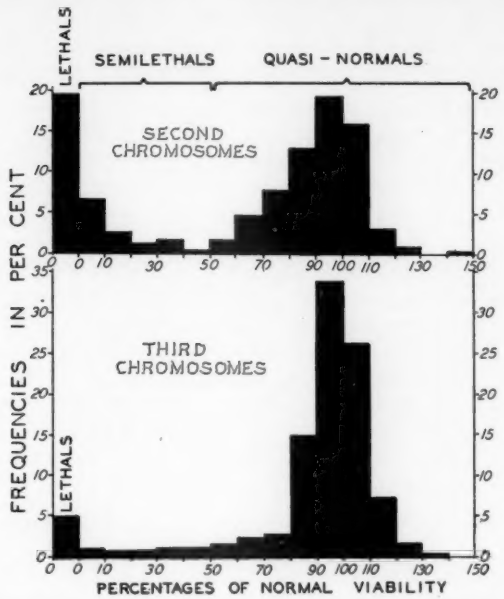

FIGURE 1.—The influence on the viability of homozygosis for different chromosomes derived from natural populations. Abscissae—percentages of the normal viability; ordinates—frequencies (in percent) of chromosomes giving different degrees of viability in homozygotes.

tistical test for homogeneity gives a chi-square of 10.5 which, for 4 degrees of freedom, has a probability of about 0.04. The results are more uniform for the third chromosomes (table 4). Here the chi-square is 7.1, which has a probability as high as 0.13.

It is interesting to compare the frequencies of lethal and semilethal chromosomes in *D. prosaltans* with those in other species of Drosophila. Tables 5 and 6 summarize the relevant data. The natural populations of *D. willistoni* have been studied by PAVAN et al. (1951), TOWNSEND (1952) and CORDEIRO (unpublished, quoted by TOWNSEND). Very extensive data on second chromosomes of *D. melanogaster* have been assembled by IVES (1946), DUBININ (1946), GOLDSCHMIDT (1951) and OLENOV (quoted by DUBININ). PROF. P. T. IVES very generously permits us to include in this summary also his unpublished data accumulated since his paper was written. Second chromosomes of *D. pseudoobscura* and *D. persimilis* have been studied by DOBZHANSKY, HOLZ and SPASSKY (1942) and DOBZHANSKY and SPASSKY (1953).

The most direct comparisons are those between *D. prosaltans* and the Brazilian populations of *D. willistoni* (tables 5 and 6), since the population samples of both species were obtained in the same territory. Both in the second and in the third chromosomes, *D. prosaltans* has significantly fewer lethals

TABLE 5

Frequencies of lethal and semilethal second chromosomes in Drosophila prosaltans and in the corresponding chromosomes of other species.

Species	Region	Chromosomes tested	Percent lethals and semilethals	Author
prosaltans	Brazil	304	32.6 ± 2.7	CAVALCANTI, DOBZHANSKY & SPASSKY
willistoni	Brazil	2004	41.2 ± 1.1	PAVAN et al.
"	Rio Grande Sul	645	28.4 ± 1.8	CORDEIRO
"	Florida	109	31.1 ± 4.4	TOWNSEND
"	Cuba	25	36.0 ± 9.5	"
melanogaster	Lincoln, Neb.	133	25.6 ± 3.8	IVES
"	Cannonsburg, Pa.	117	28.2 ± 4.2	"
"	Syossett, N. Y.	449	31.8 ± 2.2	"
"	Monroe, N. Y.	78	34.6 ± 5.4	"
"	Amherst, Mass.	3549	36.3 ± 0.8	"
"	Pullman, Wash.	138	39.1 ± 4.2	"
"	Austin, Texas	98	41.8 ± 5.0	"
"	Wooster, Ohio	343	43.1 ± 2.6	"
"	Blacksburg, Va.	805	43.0 ± 1.7	"
"	Winter Park, Fla.	468	61.3 ± 2.3	"
"	Israel	243	38.7 ± 3.1	GOLDSCHMIDT
"	Crimea	1630	24.8 ± 1.1	DUBININ
"	Ukraine	2700	24.3 ± 0.8	OLENOV
"	South Caucasus	2738	18.7 ± 0.8	DUBININ
"	North Caucasus	795	12.3 ± 1.2	"

than *D. willistoni*. This is not unexpected, since DOBZHANSKY, SPASSKY and SPASSKY (1953) found that the spontaneous mutation rates producing lethals are, under similar controlled laboratory conditions, roughly twice as high in *D. willistoni* as they are in *D. prosaltans*. This disparity of the mutation rates is, however, too small to account for the observed disparity of the lethals accumulated in natural populations. Doubling of the mutation rate would, other genetic and environmental parameters remaining constant, increase the accumulation of lethals by at most a factor of $\sqrt{2} = 1.41$. This is sufficient to account for the observed difference in the second chromosomes (table 5),

TABLE 6

Frequencies of lethal and semilethal third chromosomes in Drosophila prosaltans and in the corresponding chromosomes of other species.

Species	Region	Chromosomes tested	Percent lethals and semilethals	Author
prosaltans	Brazil	284	9.5 ± 1.7	CAVALCANTI, DOBZHANSKY & SPASSKY
willistoni	Brazil	1166	32.1 ± 1.4	PAVAN et al.
"	Florida	122	32.8 ± 4.2	TOWNSEND
"	Cuba	39	25.6 ± 6.9	TOWNSEND
pseudoobscura	California, Yosemite	109	33.0 ± 4.5	DOBZHANSKY & SPASSKY
"	California, San Jacinto	326	21.3 ± 1.8	DOBZHANSKY, HOLZ & SPASSKY
persimilis	California, Yosemite	106	25.5 ± 4.2	DOBZHANSKY & SPASSKY

but clearly insufficient to explain the scarcity of lethals in the third chromosomes of *D. prosaltans* (table 6).

The situation is much complicated by variations in the magnitude of the accumulated stores of lethals and semilethals in different populations of the same species. In the United States, IVES found values as low as 25.6 ± 3.8 percent and as high as 61.3 ± 2.3 percent in different populations of *D. melanogaster* (table 5). There is a clear suggestion in his data of a north-south gradient, the values increasing southward. This gradient may perhaps be ascribed to the environment, particularly to temperature, since the mutation rates are well known to increase at higher temperatures. However, DUBININ and OLENOV found extremely low values in Russian populations, particularly in the Caucasus, which has a warm temperate to subtropical climate. GOLDSCHMIDT's value for Israel (Jerusalem) is close to IVES's figure for Massachusetts. The extremely low figures of DUBININ may not be comparable to those of other investigators, since they may include only the complete lethals but not the semilethals. Even so, his figure of 12.3 ± 1.2 percent for the populations of North Caucasus seems much too low.

In *D. willistoni*, the figures obtained by TOWNSEND for the second chromosomes of the Florida populations, and by CORDEIRO for the populations of the state of Rio Grande do Sul, are significantly lower than that of PAVAN et al. for tropical Brazil (table 5). This may, again, be ascribed to climatic influences, or else to the geographically marginal populations of a species having lower equilibria for accumulation of deleterious recessives (TOWNSEND 1952). TOWNSEND's failure to detect a corresponding difference for the third chromosomes of *D. willistoni* (table 6) may be due to sampling errors. The frequencies of lethals and semilethals in the second chromosomes in *D. pseudoobscura* and *D. persimilis* are as high as, or slightly lower than, in the homologous third chromosomes of *D. willistoni*, and twice to thrice as high as in *D. prosaltans* (table 6). Our figure for *D. prosaltans* is by far the lowest obtained for this chromosome in any species.

The second chromosomes of *D. prosaltans, D. willistoni,* and *D. melanogaster* are largely homologous, while the corresponding loci are distributed among the third and the fourth chromosomes in *D. pseudoobscura* and *D. persimilis* (SPASSKY, ZIMMERING and DOBZHANSKY 1950; SPASSKY and DOBZHANSKY 1950). DOBZHANSKY and SPASSKY (1953) found that about 25 percent of the third, and 26 percent of the fourth, chromosomes of the populations of *D. pseudoobscura* in the Yosemite region of California are lethal or semilethal when homozygous. The corresponding figures for *D. persimilis* from the same region are about 23 and 28 percent. Assuming that the probabilities of occurrence of lethals in different chromosomes are independent, one may estimate that, in the examined populations of *D. pseudoobscura* and of *D. persimilis*, about 45 percent of the flies carry at least one lethal or a semilethal in either the third or in the fourth chromosomes. This figure can, then, be compared to the observed values for the second chromosomes of the other species (table 5). It is higher than that found in *D. pro-*

saltans, about as high as in the Brazilian populations of *D. willistoni*, and in the midrange of values recorded by IVES for the American populations of *D. melanogaster*. Unfortunately, no data are available in the literature for the third chromosomes of *D. melanogaster*.

MEAN VIABILITY OF THE HOMOZYGOTES

Table 7 shows the mean viability of homozygotes for second, or for third, chromosomes from natural populations of certain species of Drosophila. The figures for *D. prosaltans* are calculated from the data in tables 3 and 4; those for *D. willistoni* are based on data in tables 2 and 3 of PAVAN et al. (1951), and those for *D. pseudoobscura* and *D. persimilis* are quoted from DOBZHANSKY and SPASSKY (1953). In all cases the calculations have been made in two ways: taking into account all the chromosomes, and considering only the quasi-normal chromosomes, i.e., those not containing lethals or semilethals. The figures show, then, the viabilities which the respective populations would have if they became homozygous for a certain class of chromosomes. It is

TABLE 7

Mean viability of homozygotes for certain chromosomes.

Species	Chromosome	All chromosomes	Quasi-normals
prosaltans	Second	62.63 ± 2.53	91.68 ± 1.04
willistoni	"	53.91 ± 0.94	86.72 ± 0.44
prosaltans	Third	88.38 ± 1.70	96.63 ± 0.79
willistoni	"	63.01 ± 1.17	88.77 ± 0.46
pseudoobscura	Second	55.22 ± 3.12	75.00 ± 1.22
persimilis	"	67.64 ± 2.90	87.94 ± 1.77

obvious that the loss of viability due to homozygosis would in all species be considerable, not only if the lethal-containing chromosomes were to become homozygous but even if some " eugenical " program succeeded in eliminating all but the quasi-normal chromosomes.

An important fact to be deduced from table 7 is, however, that the loss of the viability owing to inbreeding and homozygosis is smaller in *D. prosaltans* than in other species. The second chromosomes of *D. prosaltans* contain mostly the same gene loci as the second of *D. willistoni*, while the third chromosomes of *D. prosaltans* and *D. willistoni* are homologous to the second chromosomes of *D. pseudoobscura* and *D. persimilis* (SPASSKY, ZIMMERING and DOBZHANSKY 1950; SPASSKY and DOBZHANSKY 1950). To put it in another way: the adaptedness of natural populations of *D. prosaltans* is dependent on heterozygosis to a lesser extent than it is in the other species.

SUBVITALS AND SUPERVITALS

It is convenient to distinguish among the quasi-normal chromosomes those which are subvital, normal and supervital when homozygous. Of course, these three classes are not discrete, and the boundaries between them can be established only by a convention. A method of estimation of the frequencies of these classes of chromosomes has been devised by WALLACE and MADDEN (1953)

TABLE 8

Observed variance (σ_{res}^2), variance due to environment and to sampling (σ_{es}^2), genetic variance (σ_r^2), and standard deviation (σ_r) among homozygotes and heterozygotes for quasi-normal chromosomes of D. prosaltans.

Chromosome	Homozygotes				Heterozygotes			
	σ_{res}^2	σ_{es}^2	σ_r^2	σ_r	σ_{res}^2	σ_{es}^2	σ_r^2	σ_r
Second	111.0	70.4	40.6	6.37	80.8	50.1	30.7	5.54
Third	127.5	62.8	64.7	8.04	110.0	74.9	35.1	5.93

and used for natural populations of *D. pseudoobscura* and *D. persimilis* by DOBZHANSKY and SPASSKY (1953). The total, or observed, variance of the viabilities of the homozygotes for quasi-normal chromosomes (σ_{res}^2) is subdivided into two components, namely variance due to environmental fluctuations and to sampling errors (σ_{es}^2), and the "real" or genotypic variance between the homozygotes (σ_r^2). The variance observed among the heterozygotes (the control experiments) is analyzed in a similar manner. The necessary calculations have been carried out from our data by MR. W. LUKE. The results are summarized in table 8.

A subvital homozygote is defined as one whose viability is at least 2 σ_r below normal. A supervital homozygote has a viability 2 σ_r or more above normal. The estimation of the frequencies of such homozygotes is discussed in the papers referred to in the preceding paragraph, and is illustrated, for the second chromosomes of *D. prosaltans*, in figure 2. It will be recalled that normal viability is defined as the average viability of heterozygotes for pairs of chromosomes taken at random from a natural population. The normal

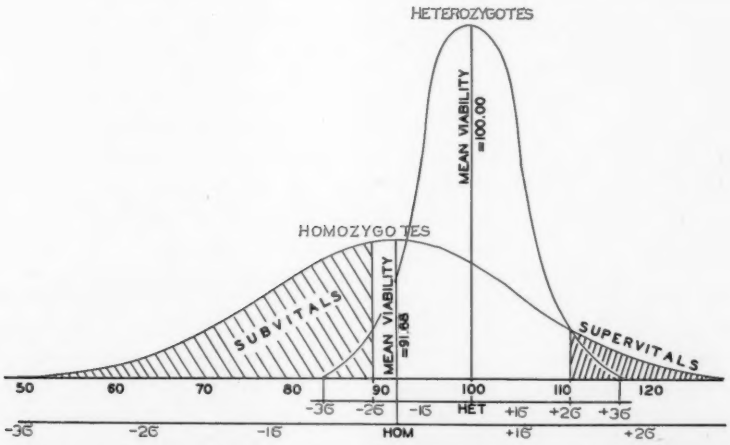

FIGURE 2.—Theoretical frequency distributions of viabilities in the homozygotes and heterozygotes for second chromosomes from natural populations of *Drosophila prosaltans*. Abscissae—percentages of the normal viability. Under the abscissa are shown the multiples of the standard deviations for the homozygotes ($\sigma_{r\ hom}$) and for the heterozygotes ($\sigma_{r\ het}$).

TABLE 9

Percentage frequencies of subvital chromosomes in three species.

Species	Chromosome	Frequency
prosaltans	Third	14.5
pseudoobscura	Second	93.5
persimilis	Second	84.4
prosaltans	Second	33.4
pseudoobscura	Third + Fourth	97.3
persimilis	Third + Fourth	99.5

viability is assigned the value of 100. Since σ_r for the second chromosome heterozygotes is 5.54 (table 8), the subvital range lies below $100 - 2 \times 5.54 = 88.92$ percent of normal viability. The mean viability of a homozygote for the second chromosome is 91.68 (table 7), and the σ_r for the homozygotes is 6.37 (table 8). The proportion of quasi-normal chromosomes which are subvital when homozygous is then estimated as the area of distribution of the homozygotes which lies below 88.92, as shown in figure 2. This turns out to be 33.4 percent of the homozygotes. The supervital homozygotes have $100 + 2 \times 5.54 = 111.08$ or more percent of normal viability (fig. 2). Their frequency is only about 0.1 percent. For the third chromosomes, the figures arrived at in a similar manner are 14.5 percent subvital and 2.9 percent supervital chromosomes. The remainder of the chromosomes (66.5 percent of the second and 82.6 percent of the third) give approximately normal viability in homozygotes.

Table 9 shows a comparison of the frequencies of subvital chromosomes in different species. The only immediately available figures are those of DOBZHANSKY and SPASSKY (1953) for the second chromosomes of *D. pseudoobscura* and *D. persimilis*, which are homologous to the third of *D. prosaltans*. It can be seen that in the two former species the subvital chromosomes are six or more times as frequent as they are in the last named species. The second chromosome of *D. prosaltans* contains most of the genetic materials borne in the third and the fourth chromosomes of *D. pseudoobscura* and *D. persimilis*. From the data of DOBZHANSKY and SPASSKY, it can be estimated that subvital second chromosomes in *D. prosaltans* are much less frequent than in the other species. Although no estimates made with the aid

TABLE 10

Percentages of chromosomes which cause sterility of the homozygotes.

Species	Chromosome	Females	Males
prosaltans	Second	9.2 ± 2.2	11.0 ± 2.4
willistoni	"	40.5 ± 5.7	64.8 ± 5.5
pseudoobscura	(Estimate)	17.5	20.8
persimilis	(Estimate)	29.5	23.6
prosaltans	Third	6.6 ± 1.8	4.2 ± 1.3
willistoni	"	40.5 ± 7.8	66.7 ± 7.4
pseudoobscura	Second	10.6 ± 3.3	8.3 ± 3.0
persimilis	"	18.3 ± 4.6	13.2 ± 4.1

of the present techniques are available for the subvitals in *D. willistoni*, the data of PAVAN et al. (1950) leave no doubt that subvital chromosomes in that species are also far more frequent than they are in *D. prosaltans*. No estimates at all exist for natural populations of *D. melanogaster*. WALLACE and MADDEN (1953) found between 37 and 45 percent subvital second chromosomes in their experimental populations of this species.

STERILITY AND OTHER GENETIC TRAITS

The homozygotes for 173 second and 213 third chromosomes were tested for fertility. The chromosomes so tested allowed quasi-normal viability in the homozygotes, although several of the less extreme semilethals (30 percent of normal viability and upwards) were included. The tests were made separately for females and for males, as indicated in PAVAN et al. (1950). In only four instances has the homozygosis for a chromosome caused sterility of both sexes; most of the chromosomes which produce sterility do so in one sex only.

The results for *D. prosaltans* are reported in table 10 together with analogous data of PAVAN et al. for *D. willistoni* and of DOBZHANSKY and SPASSKY (1953) for *D. pseudoobscura* and *D. persimilis*. As explained above in connection with the comparisons of the frequencies of lethal and viability genes in these species, the third chromosome of *D. prosaltans* corresponds to the third of *D. willistoni* and to the second of *D. pseudoobscura* and *D. persimilis*. The second chromosome of *D. prosaltans* is homologous to the second of *D. willistoni*, and to the sum of the third and the fourth chromosomes of *D. pseudoobscura* and *D. persimilis*. For the purpose of comparison, the frequencies of *D. pseudoobscura* and *D. persimilis* which carry sterility factors either in their third, or in their fourth, or in both chromosomes have been estimated from the data of DOBZHANSKY and SPASSKY on the assumption of independent occurrence of such sterility factors.

Table 10 shows that the sterility of the homozygotes is least frequent in *D. prosaltans*, more so in *D. pseudoobscura* and *D. persimilis*, and most frequent in *D. willistoni*.

Our experimental design is unfavorable for the detection of variations in the development rates of the homozygotes, since, as shown in table 2, the marking genes used cause a pronounced retardation of the development of their carriers. Inspection of the data demonstrates beyond doubt that some of the homozygotes for the second as well as for the third chromosomes develop more slowly than do others, but estimation of the frequencies of such homozygotes would be too laborious as well as insufficiently reliable. Some of the homozygotes proved to possess morphological abnormalities which make them recognizable by inspection.

DISCUSSION

The deleterious effects of homozygosis for chromosomes derived from natural populations of *Drosophila prosaltans* are less severe on the average than is the case in other species—*D. willistoni, D. melanogaster, D. pseudo-*

obscura and *D. persimilis*. This conclusion emerges quite clearly, despite some inconsistencies in the data, due in part to sampling errors, in part to the variability observed among geographic populations of the same species, and in part to unexplained but real differences in behavior of the corresponding chromosomes in different species.

The mutation rates producing lethals and semilethals are lower in *D. prosaltans* than in other species (DOBZHANSKY and SPASSKY 1952). This fact does not account, however, for relative resistance of *D. prosaltans* to inbreeding depression. It is probable that the low mutation rates as well as the relative weakness of heterosis in this species are both consequences of a more fundamental cause. This cause may be found in the ecological nature of the species. In at least the Brazilian part of its distribution area, *D. prosaltans* rarely forms large and dense populations; it usually occurs in "spots," and is, except in few localities which are evidently favorable to it, a rare species. Although we do not know just what ecological specializations are responsible for the rarity and the irregular spatial occurrence of *D. prosaltans*, these specializations profoundly influence the reproductive biology and, consequently, the genetic structure of the species.

In natural populations of common, successful and ecologically versatile species, such as *D. willistoni* and *D. pseudoobscura*, most chromosomes (autosomes) carry gene complexes which are more or less severely deleterious in homozygous condition. This is presumably also true in natural populations of *D. melanogaster*; at any rate WALLACE and MADDEN (1953) have found such a situation developing in their experimental populations in the laboratory. Since in large populations any one chromosome is rarely present in duplicate (in homozygous condition) in any individual, the fitness of homozygotes is of relatively little importance for the fitness of a large population as a whole. Natural selection acts rather to promote formation of a gene pool containing numerous alleles at many loci, and even more numerous linked gene complexes, which yield high fitness in heterozygotes with other alleles and gene complexes present in the same population. Geographically remote populations may, however, have differently organized gene pools, with gene complexes not coadapted to each other. This accounts for the breakdown of fitness caused by the gene recombination in interpopulational hybrids, despite an ephemeral luxuriance in the F_1 generation (VETUKHIV 1953; BRNCIC 1954). Ecological success of biotically versatile species leads, then, to increasing importance of heterozygotes. *Mutatis mutandis*, this kind of genetic population structure permits the species to become more versatile and more successful. The genetic structure and the ecological nature of a species are interdependent.

There is, however, enough place in nature for rare species the welfare of which depends upon exploitation of more specialized ecological opportunities. *D. prosaltans* appears to be one of the species which has embarked on such a career. Here the adaptedness of homozygotes as well as of heterozygotes is important. The result is that a compromise is struck: gene alleles and gene

combinations which are favored by natural selection give reasonably high fitness both when heterozygous and when homozygous. Of course, this does not eliminate the occurrence of mutations which are useless under all environmental conditions. The mutation rates are merely reduced to a minimum which is still compatible with preservation of evolutionary plasticity. There is again a reciprocity and interdependence between the ecology and the genetic population structure.

It is interesting that population structures exemplified by *D. prosaltans* on one hand and *D. willistoni* and *D. pseudoobscura* on the other, occur within the same genus and subgenus. How wide is the occurrence of these, intermediate, and more extreme population structures in other organisms can hardly be conjectured at present. CROW (1952, and earlier) has shown that the heterotic state in maize is very largely dependent upon coadapted gene alleles or gene complexes. LERNER (1954a, b) finds evidence that coadaptation is important in populations of poultry and of other domestic animals. It would, however, be desirable to compare the amounts of inbreeding depression in different species and in different breeds within a species, especially where the degree of inbreeding is high and the genetically effective populations are small. Normally self-fertilized crops, such as wheat, are at the opposite end of the spectrum compared to *D. willistoni*; in them the importance of fitness of homozygotes is paramount. By far the most vital problem in this field is, of course, the situation of the human species; without knowing more than we do about this situation no satisfactory evaluation of "Our load of mutations" (MULLER 1950) is possible.

SUMMARY

Drosophila prosaltans is a species which occurs in the American tropics from the West Indies to southern Brazil. In most places, at least in Brazil, this species is rather rare, found chiefly in isolated pockets, and apparently restricted to some specialized ecological niches. The genetic variability carried in natural populations of this species has been studied and compared with that known in other species, especially in *D. willistoni*. The last named species occurs in the same gross territory as *D. prosaltans*, but it is ubiquitous, very common, and apparently ecologically versatile.

As in other species of Drosophila, natural populations of *D. prosaltans* carry in heterozygous condition numerous recessive lethals, semilethals, subvitals, sterility genes, visible mutants, and other deleterious genetic variants. However, the deleterious effects of inbreeding are less severe in *D. prosaltans* than they are in *D. willistoni* and in other species. The small proportion in *D. prosaltans* of chromosomes which are subvital when homozygous is especially noteworthy.

The relative unimportance of heterosis in *D. prosaltans* is probably related to the ecological nature of this species. In a species which exists mostly in small and isolated populations, the effects on fitness of genes and gene complexes both in homozygous and in heterozygous condition are important. By

contrast, in common and ecologically dominant species, the effects of an allele, or of a gene complex in heterozygotes with other alleles and complexes present in the same population are most important for natural selection. The genetic structure and the ecological characteristics of a living species are interrelated.

ACKNOWLEDGMENTS

The collecting of the material for the present study has been made possible by grants given by the Rockefeller Foundation (to Columbia University) and by the Conselho Nacional de Pesquisa (to the University of São Paulo) to defray the cost of the collecting in various parts of Brazil, and by the hospitality and cooperation of several colleagues, especially Drs. A. Brito da Cunha and C. Pavan of the University of São Paulo, A. G. L. Cavalcanti of the National University at Rio de Janeiro, G. Addison, F. Camargo, R. L. Froes, Rubens Lima and J. Murça Pires of Instituto Agronomico do Norte at Belem, Agostinho Reies of Floriano, and the members of the family Pena of the Isle of Marajo. Miss Sophie Dobzhansky has participated in the collection of the material, while Mrs. N. Spassky, Olga Pavlovsky and N. Dobzhansky shared in some of the work of raising and counting the flies. Drs. H. Levene, Bruce Wallace and Mr. R. Lewontin gave valuable advice on statistical procedures, while Mr. W. B. Luke made many of the calculations. Dr. P. T. Ives of Amherst College and Prof. A. R. Cordeiro of the University of Porto Alegre, Brazil, have generously permitted the use of their unpublished data for comparative purposes.

LITERATURE CITED

Brncic, D., 1954 Heterosis and the integration of the genotype in geographic populations of *Drosophila pseudoobscura*. Genetics **39**: 77–88.

Cavalcanti, A. G. L., 1950 Contribuição a genetica das populações naturais: analise dos gens autossomiscos recessivos dos chromossomos II e III de *Drosophila prosaltans* Duda. Tese da Facul. Nac. Filosofia, Univ. Brazil, 98 pp., Rio de Janeiro.

Crow, J. F., 1952 Dominance and overdominance. In "Heterosis," Iowa State College Press: 282–297.

Dobzhansky, Th., A. M. Holz, and B. Spassky, 1942 Genetics of natural populations. VIII. Genetics **27**: 463–490.

Dobzhansky, Th., and C. Pavan, 1950 Local and seasonal variations in relative frequencies of species of Drosophila in Brazil. J. Anim. Ecol. **19**: 1–14.

Dobzhansky, Th., and B. Spassky, 1953 Genetics of natural populations. XXI. Concealed variability in two sympatric species of Drosophila. Genetics **38**: 471–484.

Dobzhansky, Th., B. Spassky, and N. Spassky, 1953 A comparative study of mutation rates in two ecologically diverse species of Drosophila. Genetics **37**: 650–664.

Dubinin, N. P., 1946 On lethal mutations in natural populations. Genetics **31**: 21–38.

Goldschmidt, E., 1951 Deleterious genes in wild *Drosophila melanogaster* from Israel. Amer. Nat. **85**: 200–205.

Ives, P. T., 1945 The genetic structure of American populations of *Drosophila melanogaster*. Genetics **30**: 167–196.

Lerner, I. M., 1954a Genetic homeostasis. Oliver and Boyd, Edinburgh.

1954b The genotype of Mendelian populations. Proc. Ninth Internat. Congress Genetics (in press).

MULLER, H. J., 1950 Our load of mutations. Amer. J. Human Genet. **2**: 111–176.

PAVAN, C., 1952 Relações entre populações naturais de Drosophila e o meio ambiente. Tese da Facul. Filosofia, Univ. São Paulo. 107 pp., São Paulo.

PAVAN, C., A. R. CORDEIRO, TH. DOBZHANSKY, N. DOBZHANSKY, C. MALOGOLOWKIN, B. SPASSKY and M. WEDEL, 1951 Concealed genic variability in Brazilian populations of *Drosophila willistoni*. Genetics **36**: 13–30.

SPASSKY, B., and TH. DOBZHANSKY, 1950 Comparative genetics of *Drosophila willistoni*. Heredity **4**: 201–215.

SPASSKY, B., S. ZIMMERING and TH. DOBZHANSKY, 1950 Comparative genetics of *Drosophila prosaltans*. Heredity **4**: 189–200.

TOWNSEND, J. I., 1952 Genetics of marginal populations of *Drosophila willistoni*. Evolution **6**: 428–442.

VETUKHIV, M. A., 1953 Viability of hybrids between local populations of *Drosophila pseudoobscura*. Proc. Nat. Acad. Sci. **39**: 30–34.

WALLACE, B., C. MADDEN, 1953 The frequencies of sub- and supervitals in experimental populations of *Drosophila melanogaster*. Genetics **38**: 456–470.

GENETICS OF HETEROSIS FOR EARLINESS IN THE TOMATO[1]

ALLAN B. BURDICK

Department of Biological Sciences, Purdue University, Lafayette, Indiana

Received December 11, 1953

EARLINESS of fruiting or of maturity is quite often an important factor in the usefulness of an agricultural crop. In the cultivated tomato, early flowering is generally associated with higher yield of ripe fruit.

In tomato hybrids, the F_1 is usually earlier than the earliest parent in flowering and in fruit setting (WELLINGTON 1922; POWERS and LYON 1941; HAYES and JONES 1917). This is similar to the behavior of hybrids in maize (JONES 1918; SPRAGUE 1936) but is a contrast to hybrids in barley (SUNESON and RIDDLE 1944), soybeans (WEBER 1950), pearl millet (BURTON 1951), onion (EMSWELLER and JONES 1935) and wheat (THOMPSON 1918). It is apparent that although heterosis may be evident in many aspects of hybrid plant development, it is not always manifest in earliness.

Probably the most dependable index of heterosis in plant hybrids is the growth rate and eventual size of the hybrid plants. If a generalization could be formulated to include all cases of heterosis, it might state that heterotic hybrids grow faster and reach a greater size at maturity than do plants of the faster growing, larger sized parent. In specific instances of heterosis, other plant characteristics may be expressed to a greater or lesser degree than the most extreme of the parents. Earliness in the tomato is such a trait.

The genetic causes of heterosis for earliness (or negative heterosis for "days to first flower") in the tomato may, conceivably, be ascribed to overdominance (HULL 1945, 1946, 1947), nicking action of genes with no more than complete dominance (JONES 1917), or to epistasis (GRIFFING 1950; BURDICK 1949). A limited method for distinguishing the most apparent among the aforementioned causes has been given by GRIFFING (1950).

MATERIALS AND METHODS

The plant materials used in this experiment consisted of eight inbred lines of tomatoes and all their combinations in hybrids, excluding reciprocals. A brief description of each of the parental lines and their experimental designations follows:

Line A. *Lycopersicon pimpinellifolium*; entry 33, seed from 47B.052.A4 which was grown from seed obtained from J. A. JENKINS in 1947, his number 152. This is the wild Red Currant tomato having, typically, many one-gram, two-locule fruits and a viney, prostrate type of growth.

[1] Supported, in part, by a predoctoral fellowship (1948–49) of the Atomic Energy Commission; carried on in the Division of Genetics, University of California, Berkeley, California.

Line B. *L. esculentum*; entry 24, seed from 47B.018.B1 from 46.018.1 which was grown from seed obtained from E. W. LINDSTROM in 1946, his number 2292.3. This is a semi-wild Red Cherry type sometimes called *cerasiforme*, having many three- to four-gram, two-loculed fruits. The fruits are larger than most *cerasiforme* types and the vine is more erect with somewhat larger leaves. It is an excellent tomato to work with in the greenhouse.

Line C. *L. esculentum*; entry 26, seed from 47B.066.C2 which was grown from seeds obtained from J. A. JENKINS in 1947, his number 841. This line carries the designation RSI (Rick's short internode—C. R. RICK, Davis, California) and has extremely short internodes, is glabrous, erect, and bushy, and has ribbed, many-loculed, ten- to twenty-gram fruits.

Line D. *L. esculentum*; entry 15, seed from 47B.095.D2 which was grown from seed obtained from J. A. JENKINS in 1947, his number 384. This line had an erect, vigorous type of vine with pear-shaped (pyriform), two-loculed, four-gram fruits. Under Berkeley conditions, it was a poor fruit-setter.

Line E. *L. esculentum*; entry 31, seed from 47B.089.E2 which was grown from seed obtained from J. A. JENKINS in 1947, his number 622. This line is known locally, where it was collected in Mexico in 1943, as criollo. It has ribbed, many-loculed, ten- to twenty-gram fruits much like line C but its growth habit is more normal, like that of typical *cerasiforme*.

Line G. *L. esculentum*; entry 25, seed from 47B.045.G1 which was grown from seed obtained from J. A. JENKINS in 1947, his number 173. This is the well-known English forcing variety, Sutton's Best-of-all (SBA). It has smooth, three- to four-loculed, 30- to 50-gram fruits with typical garden variety tomato growth habit. It is an excellent fruit-setter under Berkeley conditions, both in the greenhouse and in the field.

Line J. *L. esculentum*; entry 32, seed from 47B.091.J which was grown from seed obtained from J. A. JENKINS in 1947, his number 623. This is a non-aggressive, decumbent type of *cerasiforme* with two-loculed, two-gram, colorless-skinned (*yy*) fruits. It was a poor fruit-setter under Berkeley conditions and the vine had a dark green, often purple, color.

Line K. *L. esculentum*; entry 6, seed from 47B.001.K, from 47.001.1, from 46.001.16. Number 46.001.16 was a diploid callus shoot obtained from a 10-year-old haploid grown by E. W. LINDSTROM. This line is a slow-growing, erect, light green type with smooth, many-loculed, 40- to 60-gram fruits. The fruit has a colorless skin (*yy*).

The 28 possible hybrids between these eight parent lines were made during the winter of 1947–48 in the greenhouse at Berkeley. Good seed set was readily obtained with hand cross pollination.

The parents and hybrids were grown together in a 6×6 triple lattice design with 6 replications in the field during the summer of 1948. The triple lattice design made possible the recovery of inter-block information where necessary. Seed for the parents was obtained, in most cases, from the plant used in the hybridizations; otherwise from a full sib of that plant. At transplanting time, each plot in the experiment contained four plants. At 90 days after germi-

nation, the plots were thinned to two plants in order to take green-weight-of-plant data. All data analyzed are based on plot means.

Plant disease was relatively light, 14 plants out of 864 being removed because of disease. Over the whole experiment, the mean position of the first fruit set was the fourth flower on the first inflorescence. Most of the hybrids set fruit on the first flower of the first inflorescence and all subsequent flowers.

Plants were hand watered at transplanting time and the whole field watered from an overhead sprinkling system at about 70 days after transplanting. A DDT dust was used to control predatory insects.

Data were obtained for 32 different plant characters. Six of these characters were considered to be direct functions of sexual earliness and constitute the basis of the report given here. These characters and the method of obtaining the data were:

S3. Days from germination to anthesis of first flower, first inflorescence. Germination data were taken on individual plants and a mean day of germination calculated. Days to anthesis were then computed from the mean day of germination for that particular line or hybrid.

TABLE 1

Analysis of covariance of S31, inflorescence and position of first ripe fruit (X) and S30, days from germination to first ripe fruit (Y).

Source	Degrees freedom	Sum of squares	Sum of cross products	(Y) Sum of squares	Errors of estimate		
					Sum of squares	Degrees freedom	Mean square
Total	215	58.9594	527.076	31841.24	27129.37	214	
Reps.	5	0.4911	2.734	275.72			
Varieties	35	39.4269	438.143	28933.95			
Error	175	19.0414	86.199	2631.57	2241.35	174	12.88
Adjusted means of S30					24888.02	40	622.20**
Regression, within varieties, of S30 on S31							b = 4.5269**

** Significant at 1% level.

S4. Days from germination to anthesis of first flower, second inflorescence. Data were computed as for S3.

S6. Days from first flower, first inflorescence to first flower, second inflorescence. Averages of S3 were subtracted from averages of S4 to give S6.

S30. Days from germination to first ripe fruit. These data were computed from the mean day of germination as for S3 and S4. In addition, days to first ripe fruit data were adjusted on the basis of the position of the first ripe fruit so that all data given represent either the actual or the expected value for a fruit ripening from the first flower, first inflorescence. The analysis of covariance of position of first ripe fruit (X) and days to first ripe fruit (Y) is summarized in table 1. The regression within varieties (b = 4.5269) indicates about a four and a half day difference between ripening of the first fruit on the first inflorescence and the first fruit on the second inflorescence. If, for instance, a variety such as B actually set the first fruit, on the average, from

the first flower on the second inflorescence, then the observed value for days to first ripe fruit for B was adjusted downward by about 4.5 days and likewise for the other hybrids and parents to make all data comparable at the first position on the first inflorescence.

S31. Inflorescence and position of first ripe fruit. The position of the first fruit on each plant was scored in such a way that a value of 1.00 indicated that fruit was set on the first flower of the first inflorescence, a value of 2.00 indicated the first position on the second inflorescence, etc. Decimal values between 1.00, 2.00 and 3.00 were based on the mean number of flowers per inflorescence of each hybrid or parent line. For instance, if a plant set its first fruit at the third flower of the first inflorescence and that variety had an average of seven flowers per inflorescence then it received a score of $1.00 + 2(1/7) = 1.29$.

S32. Days from first flower to first ripe fruit. Mean values for S3 were subtracted from mean values for S30 to give mean days from first flower to first ripe fruit.

The method of analysis applied to these data has been given in detail by GRIFFING (1950) along with possible interpretations. In addition, the mean of all F_1's $(\overline{x}_1 + \overline{x}_2 + \ldots + \overline{x}_n/n)$ is compared with the complete potence estimate, $\overline{F}_{II}$ or $\overline{F}_{III}$ for a general indication of heterosis. (Potence, according to WIGAN 1944, is a reflection of dominance or epistasis or both but does not include metrical basis. A hybrid having the value of either parent shows a potence of 100. A hybrid with a value exactly intermediate between the two parents has a potence of zero.) The expectation of the grand mean of $\binom{n}{2}$ F_1's in terms of the grand mean of n parents under three conditions of potence is as follows:

Let: L_i = the value of the i^{th} parent
n = number of parents
$\overline{F}$ = the grand mean of $\binom{n}{2}$ F_1's
$\overline{P}$ = the grand mean of n parents
F_{ij} = the mean of the F_1 produced by crossing the i^{th} and j^{th} parents.

Case I. This is the condition of no potence, where each F_1 has a value equal to the arithmetic mean of its parent values.

Then: $F_{ij} = (L_i + L_j)/2$
Also: $\overline{P}_I = (L_a + L_b + \ldots + L_n)/n$

$$\text{And: } \overline{F}_I = \frac{[(L_a + L_b)/2] + [(L_a + L_c)/2] + \ldots + [(L_{n-1} + L_n)/2]}{n(n-1)/2}$$

$$= (L_a + L_b + \ldots + L_n)/n$$

Therefore: $\overline{F}_I = \overline{P}_I$ (1)

Case II. This is the case where potence of the gamete from the smaller parent is complete in every cross.

Then: $F_{ij} = L_i$ where $L_a < L_b < \ldots < L_i < L_j < \ldots < L_n$
As before: $\bar{P}_{II} = (L_a + L_b + \ldots + L_n)/n$

$$\text{And: } \bar{F}_{II} = \frac{F_{ab} + F_{ac} + \ldots + F_{bc} + F_{bd} + \ldots + F_{(n-1)n}}{n(n-1)/2}$$

$$= 2\left[\left(\frac{L_a + L_b + \ldots + L_n}{n-1}\right) - \left(\frac{L_a + 2L_b + \ldots + nL_n}{n(n-1)}\right)\right]$$

However, since: $\bar{P}_{II} = (L_a + L_b + \ldots + L_n)/n$

Therefore:

$$\bar{F}_{II} = 2\left[\bar{P}_{II} + \frac{(L_a + L_b + \ldots + L_n) - (L_a + 2L_b + \ldots + nL_n)}{n(n-1)}\right] \quad (2)$$

Case III. This is the case where potence of the gamete from the larger parent is complete in every cross.

Then: $F_{ij} = L_j$ where $L_a < L_b < \ldots < L_i < L_j < \ldots < L_n$

And by the method used in Case II:

$$\bar{F}_{III} = 2\left[\bar{P}_{III} + \frac{(L_a + L_b + \ldots + L_n) - (L_n + 2L_{n-1} + \ldots + nL_a)}{n(n-1)}\right] \quad (3)$$

The coefficient b_2 is calculated by regression of the constant parent regression coefficients on the associated parent values. If the mean of F_1's is less than $\bar{F}_{II}$, it can be shown that under certain circumstances b_2 is positive when (negative) dominance is the cause of heterosis. If the mean of F_1's is greater than $\bar{F}_{III}$, then b_2 will be negative when there is dominance. $\hat{X}$ is calculated from the regression equation with b_2 when $\hat{Y}$, an estimate of the constant parent regression coefficient (b), is equal to zero. $\hat{X}$ is an estimate of the value of the most potent parent. If the parents include the " best possible homozy-

TABLE 2

Correlations (r) from arithmetic data between means and variances of plot means within parent lines for various earliness characteristics.

Characteristic	r	Scale used in analysis
S3. Days to first flower on first inflorescence	−.17	Arithmetic
S4. Days to first flower on second inflorescence	.69	Arithmetic
S6. Days between first flower, first inflorescence and first flower, second inflorescence	.45	Arithmetic
S30. Days to first ripe fruit	−.21	Arithmetic
S32. Days from first flower to first ripe fruit	−.02	Arithmetic
S31. Position of first ripe fruit	.69	Arithmetic

gote " and parents show a mean in excess of $\hat{X}$ in a trait involving dominance, it is an indication that all of the heterosis shown can be accounted for by genes exhibiting no more than complete dominance (i.e., not overdominant).

Coefficients of correlation of plot means and plot variances within parent lines were calculated and are given in table 2. A significant correlation between means and variances would indicate the need for a transformation of the data to units of measurement giving no mean-variance correlation. No significant correlations were found in the earliness data when an arithmetic scale of days was used, so no transformations were made.

S3. Days to first flower on first inflorescence

Data on S3 were taken daily in the field and means were computed from the mean day of germination of each line or hybrid. These data are given in table 3 along with the analysis of variance. The grand mean of all F_1's and the grand mean of parents are about the same being 57.38 and 57.25 days respectively. The value $\overline{F}_{III}$ is an estimate of what the mean of all F_1's would have been had the later parent been completely potent in each cross. Had there been a general manifestation of heterosis for lateness, the grand mean of F_1's

TABLE 3

S3. Mean days from germination to first flower, first inflorescence.

Analysis of variance

Source	Degrees of freedom	Mean square
Replications	5	18.85**
Varieties	35	213.43**
Error	175	3.19

** Significant at 1% level.

Means

	A	B	C	D	E	G	J	K	CPS*
A	47.83	53.46	53.83	53.79	44.46	56.50	46.71	59.63	52.63
B		59.71	57.17	62.92	58.04	60.88	56.29	63.08	58.83
C			57.13	53.33	48.71	61.38	52.29	63.42	55.73
D				67.88	56.29	61.54	63.79	66.08	59.68
E					47.54	51.79	52.28	63.92	53.64
G	Mean of F_1's		57.38			57.38	60.96	61.04	59.15
J	Mean of parents		57.25				54.96	63.13	56.49
K	$\overline{F}_{III}$		61.64					65.54	62.90

The eight parent lines are indicated by letters across the top and along the left hand side of the table. Parent means are given on the upper-left-to-lower-right diagonal while the hybrid means may be found at the intersection of the line and column of the two parents involved.

* Constant parent series mean.

00.00 Earliness heterosis.

00.00 Lateness heterosis.

would be expected to exceed $\overline{F}_{III} = 61.64$. The data give no indication of a general heterosis for earliness or lateness at this stage of plant development. However, three of the hybrids were later than the latest one of their parents and four of them were earlier than the earliest of their parents.

S4. Days to first flower on second inflorescence

At the time of flowering of the first inflorescence (S3) there was very little indication that the hybrids would be heterotic for earliness. In fact, had the trends indicated in individual hybrids gone on to materialize in heterosis later on, they would have produced an assortment of types, some heterotic

TABLE 4

S4. Days from germination to first flower, second inflorescence.

Analysis of variance

Source	Degrees of freedom	Mean square
Replications	5	10.95**
Varieties	35	137.88**
Error	175	1.77

** Significant at 1% level.

Means

	A	B	C	D	E	G	J	K	CPS*
A	58.08	62.29	62.96	63.79	56.65	65.71	56.50	68.00	62.27
B		66.63	65.17	69.79	66.21	69.00	63.88	71.33	66.81
C			66.50	65.08	62.71	70.46	63.33	70.63	65.76
D				74.04	65.71	70.33	70.75	73.33	68.40
E					62.38	66.00	62.69	71.67	64.52
G	Mean of F_1's		66.59			74.69	69.17	71.13	68.83
J	Mean of parents		67.58				63.21	70.38	65.24
K	$\overline{F}_{II}$		63.76					75.13	70.92

* Constant parent series mean.
00.00 Earliness heterosis.

for lateness and some heterotic for earliness. Nevertheless, when data for S4, days to first flower on second inflorescence, are obtained, the trend toward greater earliness in the hybrids is evident. Table 4 gives these second inflorescence data. At this stage the F_1's are about a day earlier than the average of the parents (66.59 days for the F_1's and 67.58 for the parents) while the complete potence estimate for earliness, $\overline{F}_{II}$, is 63.76, still indicating no general heterosis. It is interesting to note that the three hybrids which were heterotic for lateness at the time of flowering of the first inflorescence (S3), now have mean flowering dates intermediate between the two parents, and that all of the F_1's which show heterosis at the second inflorescence, show heterosis for earliness.

S6. Days between first flower on first inflorescence and first flower on second inflorescence

These data (table 5) were obtained by subtracting the value for S3 from that of S4 for each plot. This character is no more than an intensification of the trend already evident in S4, namely the tendency toward earlier maturity in the F_1's. The grand mean of F_1's is 9.22 and for parents is 10.37 whereas the estimate, $\overline{F}_{II}$, for complete potence of earliness is 8.14. The obtained value for F_1's (9.22) lies about mid-way between that expected with no potence ($\overline{F}_I = 10.37$) and that expected with complete potence of earliness ($\overline{F}_{II} = 8.14$). These means also show a great many more individual cases of

TABLE 5

S6. Days between first flower, first inflorescence and first flower, second inflorescence.

Source	Degrees of freedom	Mean square
Replications	5	2.15
Varieties	35	37.44**
Error	175	.96

** Significant at 1% level.

Means

	A	B	C	D	E	G	J	K	CPS*
A	10.25	8.83	9.13	10.00	12.24	9.21	9.95	8.38	9.68
B		6.92	8.00	6.88	8.17	8.13	7.58	8.25	7.98
C			9.38	11.75	14.00	9.08	11.04	7.21	10.03
D				6.17	9.42	8.79	6.96	7.25	8.72
E					14.83	14.10	10.42	7.75	10.87
G	Mean of F_1's		9.22			17.56	8.21	10.08	9.66
J	Mean of parents		10.37				8.25	7.25	8.77
K	$\overline{F}_{II}$		8.14					9.58	8.02

* Constant parent series mean.
00.00 Earliness heterosis.
00.00 Lateness heterosis.

earliness heterosis. However, two F_1's, CD and CJ, show heterosis in the direction of lateness. At the time of flowering of the first inflorescence (S3), both CD and CJ show earliness heterosis. These two hybrids must contain a particular combination of genes which makes them unusually early at the time of first flower (only two other hybrids were heterotic for earliness at the first inflorescence) and unusually slow in development during the period from first to second inflorescence (these were the only two hybrids to show lateness heterosis in S6). This appears as though CD and CJ might be producing more flowers per inflorescence but this is not so. The mean of the entire experiment was 12.9 flowers per inflorescence and CD and CJ had means of 13.2 and 12.5, respectively. Certainly, S3 and S6 are measures of

the same type of physiological activity (earliness of flowering) but involve different growth stages. S3 covers the ca. 55-day period from germination to first flower and S6, the next ca. 10-day period until the first flower blooms on the second inflorescence. The unusual aspect of the behavior of CD and CJ is that they must undergo a complete reversal of developmental rate, relative to the other hybrids in this experiment, during a period of no more than 10 days. This would seem to be a rather clear indication of the specificity of genic activity in a polygenic characteristic.

S30. Days from germination to first ripe fruit

The number of days from germination until the first fruit ripened was recorded for each plant along with the inflorescence and position of the first ripe fruit (S31). A covariance analysis of S31 and S30, table 1, gave a correction factor, b = 4.5269, the regression coefficient within varieties of S30 on S31, which was used to adjust the actual S30 data to the first position on

TABLE 6

S30. Days from germination to first ripe fruit.

	Means									
	A	B	C	D	E	G	J	K	CPS*	CPR**
A	98.5	103.7	107.1	103.8	94.3	103.5	93.6	104.9	101.6	0.4475
B		124.4	125.5	123.8	120.4	123.7	109.0	125.5	118.8	0.5504
C			129.6	115.9	112.7	122.7	106.4	128.3	116.9	0.5521
D				129.5	117.3	122.2	114.2	126.5	117.7	0.4483
E					109.4	107.8	105.2	119.9	111.1	0.5761
G	Mean of F_1's		113.6			128.1	108.5	126.9	116.5	0.6475
J	Mean of parents		120.2	$b_2 = 0.00490$			105.7	108.6	106.5	0.3979
K	$\overline{F}_{II}$		112.2	$\bar{X} = 9.82$				136.1	120.1	0.7009

*Constant parent series mean.
**Constant parent regression coefficient.
00.00 Earliness heterosis.

the first inflorescence by the expression, (S30) − 4.5269 [(S31) − 1]. The adjusted S30 data are given in table 6; S31 data in table 8.

The trend which was first indicated at flowering of the second inflorescence (S4) becomes quite apparent here at fruiting time. The mean of F_1's, 113.6, closely approaches its estimate with complete potence of earliness, $\overline{F}_{II}$ = 112.2, while the mean of parents is 120.2. The fruiting date of every hybrid either closely approaches that of the earlier parent or surpasses it.

A constant parent regression analysis was performed with these data yielding a b_2 value of 0.00490. According to Griffing (1950), b_2 can have a positive value when negative dominance is the principal cause of heterosis. In this case dominance of earliness (negative dominance) seems to be the reasonable explanation.

The expected value of the earliest parent with the assumption of no overdominance, $\hat{X} = 9.82$, is far beyond the range of parents used, consequently the degree of dominance necessary to explain the observations is relatively low.

S32. Days from first flower to first ripe fruit

It was evident from S3 that the hybrids held no advantage at the time of flowering on the first inflorescence. They were slightly earlier at the second inflorescence (S4) due principally to an advantage in fewer days between flower dates of the first and second inflorescences (S6). It is evident from this, then, that the earliness heterosis of S30, the time from germination to first ripe fruit, occurs entirely in the period from first flower to first ripe fruit. S32 (table 7) gives data on this period and may be expected to show some-

TABLE 7

S32. Days from first flower to first ripe fruit.

	Means									
	A	B	C	D	E	G	J	K	CPS*	CPR**
A	50.67	50.24	53.27	50.01	49.84	47.00	46.89	45.27	48.93	0.0676
B		64.69	68.33	60.88	62.36	62.82	52.17	62.42	59.97	0.6352
C			72.47	62.57	63.99	61.32	54.11	64.88	61.21	0.5373
D				61.62	61.01	60.66	50.41	60.42	57.99	0.5364
E	Mean of F_1's		56.26		61.86	56.01	52.92	55.98	57.44	0.3748
G	Mean of parents		62.92			70.72	47.54	65.86	57.31	0.7991
J	$\overline{F}_{II}$		57.88				50.74	45.47	50.01	0.0713
K	b_2		0.033					70.56	57.19	0.9735
	$\hat{X}$		47.92							

*Constant parent series mean.
**Constant parent regression coefficient.
00.00 Negative heterosis.

what more heterosis than S30. These data were obtained by subtraction of the means, S30 – S3 = S32.

In S32 the mean of F_1's, 56.26, is earlier than would be expected with complete potence of earliness, $\overline{F}_{II} = 57.88$, while the mean of parents is 62.92. Every hybrid having G or K as one of its parents shows earliness heterosis while only two hybrids with C show earliness heterosis, these being, of course, the hybrids with G and K. The C parent is the latest one in this group, with G and K next in order of lateness. The explanation of why two of the latest parents are so potent for earliness heterosis might involve overdominance, or could be explained by two recessive genes for lateness, at different loci in G than in K.

The constant parent regression analysis of these data is interesting since it reveals a high b_2 value of 0.033. By the same reasoning as was given for S30, this appears to be due to dominance of earliness. The estimate of the earliest parent from the CPR analysis is $\hat{X} = 47.92$. This is not much earlier

than 50.67, the value for parent A, and probably indicates, along with the positive b_2 value, almost complete dominance of the polygenes controlling earliness during this period. If one grants that parent A is not the earliest parent possible, then perhaps $\hat{X} = 47.92$ could be achieved with the genes available in these parents. A possible source of such a parent is indicated in the hybrid AK which has a value of 45.27.

The fact that $\hat{X}$ lies so close to the value of the earliest parent together with the fact that G and K, two of the three latest parents, are so potent for earliness suggests that overdominance may be causing heterosis in this trait but could not be detected in the analysis because of the limited range in the parents.

S31. Position of first ripe fruit

The system of giving numerical evaluation to the inflorescence and position of the first ripe fruit has been given under Materials and Methods. The integer indicates the inflorescence and the decimal, the flower on that inflorescence as a fraction of the total number of flowers present.

TABLE 8

S31. Inflorescence and position of the first ripe fruit.

	Means									
	A	B	C	D	E	G	J	K	CPS*	CPR***
A	1.04	1.12	1.08	1.06	1.00	1.04	1.00	1.02	1.05	−0.0151
B		2.05	1.57	1.63	1.63	1.39	1.80	1.44	1.51	0.1911
C			1.73	1.06	1.16	1.07	1.06	1.16	1.17	0.0407
D				1.32	1.30	1.10	1.15	1.17	1.21	0.0533
E	Mean of F_1's		1.23		1.41	1.07	1.67	1.07	1.27	0.2690
G	Mean of parents		1.76			1.20	1.29	1.17	1.16	0.1341
J	$\overline{F}_{II}$		1.36				3.12	1.03	1.29	0.1372
K	$\hat{X}$		−3.82					2.23	1.15	0.0075
	b_2		0.0183							

*Constant parent series mean.
***Constant parent regression coefficient.
0.00 Negative heterosis.

Nineteen of the 28 hybrids show earliness heterosis in S31 (table 8). Furthermore, the mean of F_1's, 1.23, is less than that expected with complete potence of early fruiting, $\overline{F}_{II} = 1.36$. CPR analysis yields a b_2 of 0.0183. The estimate of the lowest parent, $\hat{X} = -3.82$, is not reasonable.

This value of 0.0183 for b_2 together with the unreasonable value of −3.82 for $\hat{X}$, provides an interesting case for interpretation. We have strong indications of earliness heterosis in the grand mean of F_1's yet b_2 must have relatively little slope because $\hat{X}$ is thrown so far out beyond the range of parents. If b_2 had been strongly positive, then heterosis could have been interpreted on the basis of dominance. If it had been severely negative in slope, epistasis

might have been the interpretation (GRIFFING 1950; BURDICK 1949). The fact that it is neither, as indicated by the low value of $\hat{X}$, leads to the tentative conclusion that both dominance and epistasis contribute materially to the manifest heterosis.

GENE ACTION IN RELATION TO HETEROSIS

Disregarding epistasis for the moment, there are two genetic explanations for the occurrence of heterosis. Both of these explanations involve dominance, the only difference between them being in the degree of dominance postulated. Discrimination between these two hypotheses to impute the principal cause seems to lie in the answer to the question, " Can the F_1 vigor be achieved (in principle) in the homozygous condition? "

A partial answer to this question has been provided by RICHEY and SPRAGUE (1931). Heterotic F_1 corn hybrids were backcrossed with selection for high yield according to convergent improvement procedure. The data show that selection was effective in recovering some of the yield genes of the non-recurrent parent which must have been dominant. Furthermore, hybrids of the two recovered inbreds yielded as much or more than the original F_1, whereas calculations show that they are, on the average, less heterozygous than the original F_1. This indicates that at least part of the heterosis observed in the original F_1 could be isolated in the homozygous condition and consequently was caused by genes with no more than complete dominance.

EAST's (1936) speculation that normal rate genes may have many normal alleles is supported by STERN and SCHAEFFER (1943) for Drosophila where at least three normal alleles (wild-type iso-alleles) of the *ci* locus have been found, distinguishable only in their temperature reactions. EAST (1936) assumes that nondefective rate genes exist in allelic series, $A_1, A_2, \ldots, A_n$, each allele differing slightly from the others in function or efficiency. (The wild-type iso-alleles in Drosophila differ in their ability to react with substrate and in their efficiency in making the transformation of substrate to product, according to STERN's hypothesis. This may be considered a difference in function since one allele produces a sufficient amount of product A only at temperature y and another allele does so only at temperature x.) EAST's postulate may be expressed as follows:

$$A^iA^i = a_i$$
$$A^iA^j = a_i + a_j - \beta$$

Where: $a_i > \beta < a_j$

This embodies the overdominance hypothesis mentioned by FISHER, IMMER and TEDIN (1932) and elaborated by HULL (1945, 1946, 1947).

JONES (1945) describes six recessive degenerative mutations that have appeared in highly inbred lines of maize. In every case when these mutant types are crossed with their homozygous normal cousins, the F_1 shows heterosis. If these mutations are single gene mutations and the inbred lines are homozygous, these are cases of single gene heterosis and would indicate overdomi-

nance as an explanation. JONES points out that the question of whether these are single gene mutations is irrelevant to the interpretation of the cause of the heterosis. He prefers an explanation involving different functions for the mutant and normal alleles. This, although he does not signify it, is an appropriate basis for the overdominance hypothesis. Similar cases have been reported in barley by A. GUSTAFSSON (1946, 1947), and in sorghum by R. E. KARPER (1930) and J. R. QUINBY and KARPER (1948).

If, in these " single gene " cases, the mutation which took place to produce a recognizable recessive ($A \rightarrow a$) also caused several nearby polygenes to mutate, some in a plus and some in a minus direction, then it is possible to explain the occurrence of heterosis in these cases with no more than complete dominance.

HULL (1945, 1946, 1947) has reviewed the experimental evidence in favor of overdominance as an explanation of heterosis in corn. The failure of ear-to-row and other types of mass selection to materially increase yields may be due to the existence of overdominance. HULL gives data which show that when the highest yielding inbred of a series is crossed with all the others, the regression of F_1 yields on the yields of the parents is negative. With complete dominance, he says, the least this regression function would be expected to be would be zero. The fact that it is negative indicates the existence of overdominance.

TABLE 9

Theoretical case where complete dominance gives an effect similar to that of overdominance. (For explanation, see text.)

Genotype of homozygous parents	Yield of parents	F_1 yields in constant parent groups			
		1	2	3	4
1. aBcDeFgHij	1	....	5	9	13
2. aBcDEFGhij	3	5	....	7	11
3. aBcDEfGhIJ	5	9	7	....	9
4. ABCdEfGhIJ	7	13	11	9	....
Constant parent regression, b		2.00	0.93	0.07	−1.00

HULL's hypothesis of overdominance is not always necessary to explain a negative regression of F_1s on parents. Table 9 gives an example in which dominance is no more than complete, but where a negative regression of F_1 yield on yield of parents is obtained when line 4, the highest valued, is crossed with all the others. Each lower case gene has the effect of − 1 on yield and each upper case gene has the effect of + 1 and is completely dominant. Each of the four parents has a common background giving a yield of + 3.

These four hypothetical parent lines may seem unique. The low line has a plus gene for each of the three minus genes in the high line, the second line can cover only two of the minus genes in the high and the third line can cover only one. This would not be expected on the basis of a random distribution and arrangement of plus and minus genes in these lines, but randomness has not been involved in the derivation of inbred lines upon which the hypothesis of overdominance is predicated. Neither is there any evidence that the arrange-

ment of genes within the chromosome is a completely random matter. Both natural and artificial selection might be expected to contrive combinations of genes which are far from random if these combinations lend a particular fitness to the population. Therefore, the theoretical consideration of heterosis must not be limited to a concept of randomness in the distribution or the arrangement of genes.

Apparently, no general theorem can be given which would, in the average situation, lead one to expect such a negative regression for the highest valued parent group although it can occur as shown (table 9). The overdominance hypothesis offers a plausible, consistent explanation for the usual occurrence of a negative regression and is therefore more acceptable. However, it should be kept in mind that complete dominance, under special circumstances, can give the same picture. It is felt that these special circumstances may involve balanced polygenic combinations (MATHER 1943) but an appropriate hypothesis and test is not apparent at the present time.

It would seem at least theoretically possible that heterosis could result from either a positive dominance or positive epistasis type of non-additive gene action. The evidence on yield of grain in corn is in favor of an explanation based on some degree of positive dominance as the principal cause. J. B. GRIFFING (1947), however, found that heterosis in number of tassel branches among " unrelated " lines of corn could not be explained satisfactorily by a dominance type of interaction and therefore must have been due, at least in part, to epistasis.

DISCUSSION

The results given here indicate that earliness is one of the principal manifestations of heterosis in the tomato. Even though certain hybrids, notably BC, BE and BJ, fail to show heterosis at any time during the process of sexual maturation, the diversity of types used as parents and the high frequency of heterosis in the hybrids makes this phenomenon appear to be of general importance in the tomato. The hybrids BC, BE and BJ, then, seem to be exceptions to the rule. The B parent could be said to have low general combining ability in the same sense that this term is used in connection with corn inbreds.

The overall comparison of maturity in hybrids and parents is given in figure 1-A. Time, as measured by the three developmental periods from germination to first ripe fruit, is charted along the abscissa while " days earlier than the mean of parents " is plotted on the ordinate. The middle (zero point) horizontal line through each graph represents the mid-parental value or mean of parents at each stage. From figure 1-A it is apparent that the " average " hybrid is about intermediate between its parents until after the first flower blooms. The hybrids go through the second stage, from first bloom to first bloom on second inflorescence, at a rate considerably above that of the mean of parents although, as a rule, not as rapidly as the earliest parent for this stage. The third period, from first bloom on second inflorescence to first ripe fruit, finds the " average " hybrid developing faster than the most rapid-maturing of its parents. This is somewhat different from the findings of

POWERS and LYON (1941) based on the four hybrids they investigated. Their hybrids were not only heterotic during the period from flowering to fruiting but were also heterotic at the time of the first bloom. Although certain of the 28 hybrids reported here did show the same developmental pattern as described by POWERS and LYON, it does not appear to be the usual thing.

That there would be quantitative exceptions to this general picture could be expected, but hybrids CJ and CD, shown in figures 1-B and 1-C, deviate in

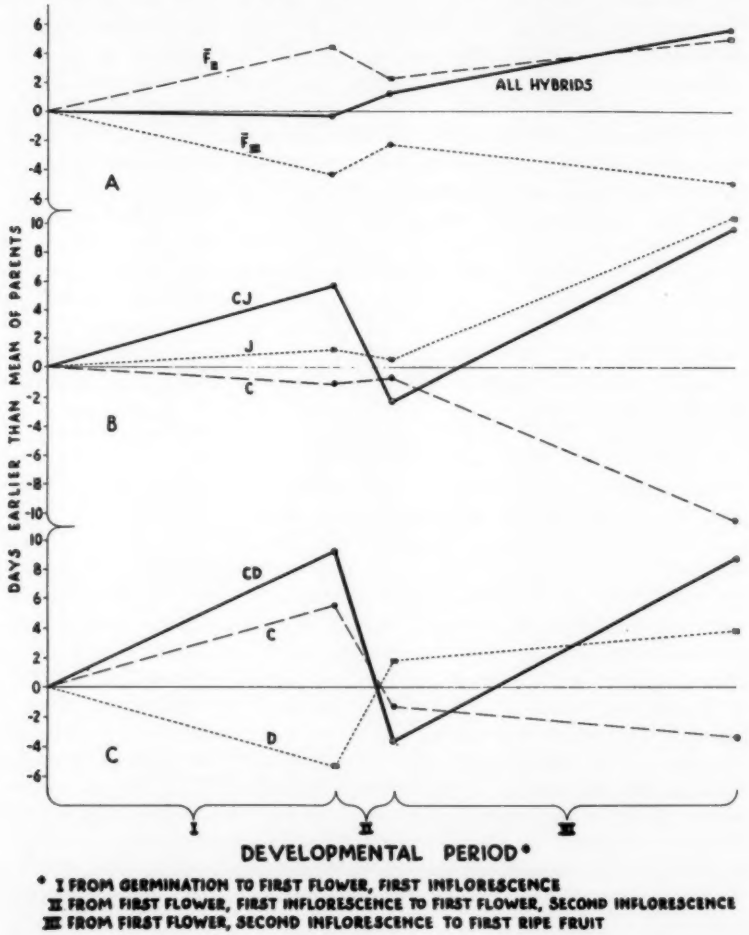

FIGURE 1.—Comparisons of earliness data taken during three different developmental periods for selected hybrids and parents and for the averages of all hybrids in relation to the complete potence estimates, $\overline{F}_{II}$ and $\overline{F}_{III}$.

TABLE 10

Summary of genetic information.

Character	Means		Potence	Phase of potence	b_3	Gene action
	F_1's	Parents				
S3. Days to first flower	57.38	57.25	2.96	Lateness		
S4. Days to first flower, second inflorescence.	66.59	67.58	25.92	Earliness		
S6. Days from first flower to first flower, second inflor.	9.22	10.37	51.11	Earliness		
S30. Days to first ripe fruit.	113.64	120.16	81.70	Earliness	0.0049	Dominance
S32. Days from first flower to first ripe fruit.	56.26	62.92	132.14	Earliness	0.0333	Dominance
S31. Position of first ripe fruit.	1.23	1.76	132.50	Earliness	0.0183	Dominance and epistasis

what might be called a qualitative way. They show atypical earliness heterosis at the time of first bloom, atypical lateness heterosis at the second inflorescence, and then a somewhat more typical developmental picture for hybrids during the last stage. It has been pointed out previously that this behavior of CD and CJ attests to the discretely different patterns of expression of heterosis which may be observed in different hybrids. If one measures heterosis only at the termination of a developmental process or only at one midpoint in the process, then these differences may not be observed.

It appears, further from figures 1-B and 1-C, that these hybrids are expressing the potence (or dominance) of one parent at one time and that of the other at another stage. If, for instance, one wished to make a statement concerning the relative dominance of C and J in the hybrid CJ, one would have to say that J was dominant at the first bloom but that C was dominant at the second inflorescence. This is not so much an enigma as it is, perhaps, a principle of dominance. BURDICK (1951) has shown in studies of kernel-row number in corn that the full parental range of row numbers may be found at different nodes in the F_1 plants. This would mean that hybrids, by virtue of having more different alleles, could call upon a broader genetic background in meeting the exigencies of development and, in cases such as the kernel-row number in corn and the maturity of the above tomato hybrids, find genes from both parents of adaptive value at different times. Here, then, may be the reason for dominance in natural populations. It provides for the independent functioning and expression of different alleles in hybrids according to the evocation of ontogenetic and environmental influences. The fact that such co-expression is not always phenotypically evident arises from the difficulty of measurement in most cases.

The data obtained in these investigations and the results of constant parent regression analyses are summarized in table 10. The potence estimates given in the table were taken from the formula:

$$\text{Potence} = \frac{100(\overline{P} - \text{mean of } F_1\text{'s})}{\overline{P} - \overline{F}_{II}}$$

where $\overline{P}$ is the grand mean of all parents and $\overline{F}_{II}$ is the complete potence estimate for earliness. In this sense, a potence of zero would result when the mean of F_1's coincides with the mean of parents and a potence of 100.0, when the mean of F_1's is equal to either $\overline{F}_{II}$ or $\overline{F}_{III}$, the complete potence estimates for earliness and lateness, respectively.

The constant parent regression analysis (b_2) in all cases indicated dominance as a principal source of non-additive genetic interaction. However, in S32 there was no way to rule out the possibility of overdominance, and in S31, it appeared as though epistatic interaction may have influenced the magnitude of b_2.

SUMMARY

It has been shown that greater earliness is one of the principal manifestations of heterosis in the tomato but that this heterosis is not evident, as a rule, until the time of the first ripe fruit.

The time of flowering in most hybrids is approximately intermediate between the flowering dates of the two parents.

Notable exceptions were found to the average earliness relationship between parents and hybrids. These exceptions indicated that even though the resulting heterosis appeared to be the same in two hybrids, their patterns of intermediate development might be quite different. The maturity genes of both parents appear to be expressing themselves, at different stages, in some hybrids. This would support the view that dominance is a relative phenomenon, depending on the stage of development and the environmental circumstances under which it is measured and that the excellence of hybrids may be attributable to the co-expression of the alleles from both parents, made possible by the existence of dominance along with ontogenetic and environmental gradients.

From a constant parent regression analysis of the data, it appears that dominance is the principal cause of earliness heterosis in the tomato.

ACKNOWLEDGMENTS

This investigation materialized after suggestions by the late E. W. LINDSTROM. It was aided by critical review and advice from J. A. JENKINS and E. R. DEMPSTER and by many helpful suggestions from J. B. GRIFFING. The author is particularly grateful for the technical help of SALLY ANN BURDICK.

LITERATURE CITED

BURDICK, A. B., 1951 Dominance, as function of within organism environment in kernel-row number in maize (*Zea mays* L.). Genetics **36**: 652–666.

1949 Heterosis in the tomato: estimation of the relative contribution of epistasis and dominance. Unpub. Ph.D. thesis, University of California, Berkeley.

BURTON, G. W., 1951 Quantitative inheritance in pearl millet (*Pennisetum glaucum*). Agron. J. **43**: 409–417.

EAST, E. M., 1936 Heterosis. Genetics **21**: 375–397.

EMSWELLER, S. L., and H. A. JONES, 1935 An interspecific hybrid in *Allium*. Hilgardia **9**: 265–273.

FISHER, R. A., F. R. IMMER and O. TEDIN, 1932 The genetical interpretation of statistics of the third degree in the study of quantitative inheritance. Genetics **17**: 107–124.

GRIFFING, J. B., 1947 Statistical analysis of components of genetic variance in maize in relation to heterosis. Unpubl. M.S. thesis, Iowa State College Library.

1950 Analysis of quantitative gene action by constant parent regression and related techniques. Genetics **35**: 303–321.

GUSTAFSSON, ÅKE, 1946 The effect of heterozygosity on variability and vigor. Hereditas **32**: 263.

1947 The advantageous effect of deleterious mutations. Hereditas **33**: 573–575.

HAYES, H. K., and D. F. JONES, 1917 The effects of cross- and self-fertilization in tomatoes. Conn. Agr. Exp. Sta. Ann. Report 1916, Part V, 305–318.

HULL, F. H., 1945 Recurrent selection for specific combining ability in corn. J. Amer. Soc. Agron. **37**: 134–145.

1946 Evidence for overdominance in corn. Oral Paper—Amer. Soc. of Agron. meetings, Omaha, Nebraska, November 1946.

1947 Evidences of overdominance in yield of corn. Genetics **33**: 110.

JONES, D. F., 1917 Dominance of liked factors as a means of accounting for heterosis. Genetics **2**: 466.

1918 The effects of inbreeding and crossbreeding on development. Bull. Conn. Agr. Exp. Sta. # 207.

1945 Heterosis resulting from degenerative changes. Genetics **30**: 527–542.

KARPER, R. E., 1930 The effect of a single gene upon development in the heterozygote in sorghum. J. Hered. **21**: 187–192.

MATHER, K., 1943 Polygenic inheritance and natural selection. Biol. Rev. **18**: 32–64.

POWERS, L., and C. B. LYON, 1941 Inheritance studies on duration of developmental stages in crosses with the genus *Lycopersicon*. J. Agr. Res. **63**: 129–148.

QUINBY, J. R., and R. E. KARPER, 1948 Effect of heterozygous condition of single gene for height of growth in sorghum. Oral Paper—Amer. Soc. Agron. meetings, Ft. Collins, Colo., August 1948.

RICHEY, F. D., and G. F. SPRAGUE, 1931 Experiments on hybrid vigor and convergent improvement in corn. Tech. Bul. USDA No. 267.

SPRAGUE, G. F., 1936 Hybrid vigor and growth rates in a maize cross and its reciprocal. J. Agr. Res. **53**: 819–830.

STERN, C., and E. W. SCHAEFFER, 1943 On wild-type iso-alleles in *Drosophila melanogaster*. Proc. Nat. Acad. Sci. **29**: 361–367.

SUNESON, C. A., and O. C. RIDDLE, 1944 Hybrid vigor in barley. J. Am. Soc. Agron. **36**: 57–61.

THOMPSON, W. P., 1918 Inheritance of the length of the flowering and ripening periods in wheat. Trans. Roy. Soc. Can. **12**(3) : 69–87.

WEBER, C. R., 1950 Inheritance and interrelation of some agronomic and chemical characters in an interspecific cross in soybeans, *Glycine max* × *G. ussuriensis*. Ia. Agr. Exp. Sta. Res. Bul. 374.

WELLINGTON, R., 1922 Comparison of first generation tomato crosses and their parents. Minn. Agr. Exp. Sta. Tech. Bul. 6.

WIGAN, L. G., 1944 Balance and potence in natural populations. J. Genet. **46**: 150–160.

TRANSLOCATIONS IN MAIZE INVOLVING CHROMOSOME 10

E. G. ANDERSON AND H. H. KRAMER

Kerckhoff Laboratories of Biology, California Institute of Technology, Pasadena,[1] *and Purdue University Agricultural Experiment Station, Lafayette, Indiana* [2]

Received December 12, 1953

THE *g-R* linkage group in maize (see EMERSON et al. 1935) was the first to be placed cytologically. MCCLINTOCK and HILL (1931) showed that the shortest chromosome of the complement was present in triplicate in plants giving trisomic ratios for *R* (aleurone color). STADLER (1933) using a deficiency, X-1, showed that the locus of *R*, but not *g* (golden plant color), was in approximately the distal quarter of the long arm. Similarly, V. H. RHOADES (1935), placed *Rp* governing resistance to race 3 of *Puccinia sorghi* in approximately the distal one-fourth of the short arm. The linkage map as given by RHOADES and RHOADES (1939) and RHOADES (1950) is

Rp	*Og*	*li*	l_8	g_1	*R*
0	16	28	38	43	57

This report will summarize the data on linkage with *g* and *R* of interchange points of 18 translocations involving chromosome 10 in relation to the cytological positions of these points as determined by LONGLEY (1950). A few tests with lineate (*li*) also are reported. Tests with *Rp*, *Og* (old-gold stripe) and l_8 (luteus-8) were not made. Similar summaries of linkage relationships of translocations have been presented by ANDERSON (1938, 1939, 1941) for chromosomes 8, 9, and the short arm of 1, and by ANDERSON and BRINK (1940) for chromosome 3. Recently, PATTERSON (1952) has summarized data for the long arm of 2 and the short arm of 9. Attention also should be called to the A-B translocations listed by ROMAN and ULLSTRUP (1951).

TRANSLOCATIONS USED AND PREVIOUS INFORMATION

The original list of ANDERSON (1935) included 16 translocations involving chromosome 10. Data on ten of these are included in this report. Translocations T 1-10b, 3-10d, 4-10a, 5-10a and 8-10d either were lost or on retesting found to involve other chromosomes. The interchange position of T 9-10a is to the right of *R*, but no data are included since special problems of survival of unbalanced gametes are involved.

Certain of the translocations have been used in previous studies. ANDERSON (1938) gave data on chromosome 9 for T 9-10b and presented data (1939)

[1] This work was supported by funds supplied by the Atomic Energy Commission through the Office of Naval Research, U.S.N., Contract N6-onr-244, Task Order 5, Project NR-164-340.

[2] Journal paper No. 769 of the Purdue University Agricultural Experiment Station.

on chromosome 8 for T 8-10a, b and c. ANDERSON and BRINK (1940) summarized chromosome 3 data of T 3-10a, b and c. ANDERSON and RANDOLPH (1945) used T 1-10a, T 2-10a, T 3-10a, T 6-10a, T 8-10a, T 8-10c and T 9-10b in establishing centromere positions on the linkage map. BURNHAM and CARTLEDGE (1939) used T 1-10a, T 3-10a and T 6-10a, in locating regions concerned with smut resistance and SABOE and HAYES (1941) used T 1-10a and T 8-10a in similar studies. BURNHAM (1949, 1950) used T 6-10a and b in studies of chromosome disjunction.

Six new translocations are reported. They were isolated from cultures used by CATCHESIDE (1938) in cytological studies following X-ray treatment of pollen and are designated by culture symbols A to K. The new translocations were crossed to a series of known interchanges and diakinesis observations of F_1 plants made following the technique of ANDERSON (1935). The origin of the lines in which chromosome 10 is involved and on which linkage data are available are:

T1-10c A50	T4-10c B45
T1-10e B98	T4-10e K17
T1-10f C36	T5-10b B70

TABLE 1

Backcross progenies from $\frac{T+R}{+g+}$, $\frac{T++}{+gR}$, $\frac{T+}{+g}$, $\frac{TR}{++}$ *and* $\frac{T+}{+R}$.

Translocation	Three-point tests					Additional two-point tests		
	Total	Parental	Recombination in region			Total	Recombinations	
			1	2	1,2		T-g	T-R
T1-10a	137	89	20	27	1	....	....	....
T1-10c	....	....	....	....	....	65	....	0
T1-10e	139	115	4	20	0	....	....	....
T1-10f	113	108	0	5	0	....	....	....
T2-10a	542	489	9	43	1	....	....	....
T3-10a	372	261	64	39	8	99	2	....
T3-10b	196	138	34	21	3	270	....	48
T3-10c	346	267	24	55	0	26	0	....
T4-10b	324	294	3	25	2	51	1	....
T4-10c	828	649	67	110	2	2015	....	387
T4-10e	....	....	....	....	....	386	....	88
T5-10b	186	108	45	27	6	66	....	17
T6-10a	274	209	26	39	0	68	7	....
T6-10b	291	236	9	45	1	236	3	....
T8-10a	357	225	81	45	6	....	....	....
T8-10a	256	202	15	37	2	....	....	....
T8-10b	461	352	42	65	2	....	....	....
T8-10c	535	347	111	66	11	....	....	....
T9-10b	135	102	21	11	1	....	....	....

LINKAGE DATA

Sixteen chromosome 10 translocations have been tested in 3-point backcross tests with *g* and *R*. The data are given in table 1. Reciprocal crossover classes were combined. Except for T8-10a, data were considered sufficiently homogeneous for combining several cultures. In several cases recombination in heterozygotes used both as males and females in backcrosses could be studied, but in the absence of marked differences, data were pooled. Additional two-point data, where available, are presented in the last three columns of table 1.

TABLE 2

Recombination percentages involving T/+, g/+ and R/+, together with cytological positions.

Translocation	Percent recombination			Cytological position†	
	T-g	g-R	T-R	Nos. 1–9	No. 10
T1-10a	15.3	20.4	34.3	1L.38	10L.21
T1-10c	....	....	0.0	1L.36	10L.67
T1-10e	2.9	14.4	17.3	1L.17	10L.30
T1-10f	0.0	4.4	4.4	1S.07	10L.65‡
T2-10a	1.8	8.1	9.6	2L.17	10L.53
T3-10a	15.7*	12.6	27.7	3L.28	10L.12
T3-10b	18.9	12.2	22.1*	3L.61	10S.25
T3-10c	6.5*	15.9	22.8	3L.27	10L.31
T4-10b	1.6*	8.3	8.6	4L.18	10L.57
T4-10c	8.3	13.5	19.8*	4S.70	10L.11
T4-10e	....	....	22.8	4L.04	10L.01
T5-10b	27.4	17.7	35.3*	5L.05	10S.24
T6-10a	9.6*	14.2	23.7	6L.68	10L.19
T6-10b	2.5*	15.8	18.6	6L.17	10L.14
T8-10a	24.4	14.3	35.3	8L.68	10S.83
T8-10a	6.6	15.2	20.3	....	....
T8-10b	9.5	14.5	23.2	8S.27	10L.14
T8-10c	22.8	14.4	33.1	8L.43	10S.51
T9-10b	16.3	8.9	23.7	9S.11	10S.28

*Includes additional two-point data.

†Longley, 1950.

‡Longley, unpublished ave. of 4 figures. Some plants give 1S.07 and 10L.26 indicating possible additional inversion in 10.

In table 2, recombination values for the *T-g*, *g-R*, and *T-R* regions are calculated using all available data from table 1. The approximate interchange positions as determined by LONGLEY (1950) are given in the last two columns of this table as the fraction of the distance from the centromere to the end of the arm involved. Generally these values are averages of three or more determinations and while accuracy to two places is not intended to be implied, the second place has been of value in the serial listing of the position of a great many translocations and has been used in calculating correlation coefficients.

Except for T1-10c where the interchange appears to be near *R*, and T1-10f

where the order is uncertain, all interchange points appear to lie to the left of *g* giving the order *T* - *g* - *R*. T2-10a and T4-10b might be questioned but the order has been confirmed by showing *g*-*R* linkage in homozygous translocation stocks. Although T4-10e has not been tested with *g*, 22.8 percent recombination with *R* and the interchange point at 10L.01 make this order likely. Data for T8-10a are inconsistent; the higher recombination values appear more in line with the cytological position on the short arm. It is possible that

TABLE 3

Backcross progenies from $\frac{T++R}{+lig+} \times +lig+$.

Gamete from F_1 female	Region of crossover	Translocation			
		T3-10a	T3-10b	T8-10a	T8-10c
T++R	0	68	68	109	40
+lig+	0	80	43	92	23
T lig+	1	1	13	1	3
+++R	1	4	3	3	9
T+g+	2	13	5	6	13
+li+R	2	12	8	5	17
T+++	3	12	11	18	11
+lig R	3	17	4	18	9
T li+R	1,2	0	1	0	0
++g+	1,2	3	1	1	10
T lig R	1,3	0	1	0	1
++++	1,3	0	1	1	1
T+g R	2,3	2	1	0	2
+li++	2,3	3	0	1	3
T li++	1,2,3	1	0	0	0
++g R	1,2,3	0	0	1	0
Sum		216	160	256	142
		Percent recombination			
T-li		4.2	12.5	2.7	16.9
li-g		15.7	10.6	5.5	31.7
T-g		16.2	20.0	6.6	34.5
li-R		26.4	20.0	19.1	43.7
T-R		27.8	27.5	20.3	43.7

the presence of an additional rearrangement such as a pericentric inversion might give the lower values observed. Both sets of values were from F_1 plants used as females in backcross.

Correlation of the interchange positions on the long arm with *T*-*g* and *T*-*R* recombination percentages gave coefficients of – .71 (9DF) and – .85 (11DF) which respectively exceed the five and one percent levels of significance. Eliminating T1-10f, 2-10a, and 4-10b where the *T*-*g* recombinations were less than two percent, a χ^2 test failed to reveal significant heterogeneity ($.3 < P > .5$) of recombination in the *g*-*R* region among the remaining 14 translocations.

TABLE 4

Classification of non-golden plants in backcross progenies of $\frac{T++R}{+lig+} \times +lig+$.

Gamete from F_1 Female	Region of crossover	Translocation	
		T6-10a	T8-10b
T++R	0	51	54
+++R	1	2	5
+li+R	2	4	5
T+++	3	6	9
T li+R	1,2	0	0
++++	1,3	0	0
+li++	2,3	0	0
T li++	1,2,3	1	0
Sum		64	73
T-li		5.9	6.9
li-g		9.8	6.9
T-g		11.8	13.7

For these translocations, therefore, there is little evidence of differential suppression of recombination in the *g-R* region. The average recombination for this region was 14.2 percent for 4063 plants.

STUDIES WITH *li*

Four-point data involving *T/+*, *+/li*, *+/g* and *R/+* for four translocations are given in table 3. The break in T3-10a appears to be to the left of *li* although the data are not conclusive. Recombination values for these cultures of T8-10a

TABLE 5

An approximation of the order from left to right of translocations involving chromosome 10.

Translocation	Recombination			Position on chromosome 10
	T-li	*T-g*	*T-R*	
T5-10b	....	27.4	35.3	S.2
T8-10c	16.9	22.8	33.1	S.5
T9-10b	....	16.3	23.7	S.3
T3-10b	12.5	18.9	22.1	S.2
T1-10a	....	15.3	34.3	L.2
T3-10a	4.2	15.7	27.7	L.1
T6-10a	5.9	9.6	23.7	L.2
T8-10b	6.9	9.5	23.2	L.1
T4-10e	....	....	22.8	L.0
T4-10c	....	8.3	19.8	L.1
T3-10c	....	6.5	22.8	L.3
T6-10b	....	2.5	18.6	L.1
T1-10e	....	2.9	17.3	L.3
T2-10a	....	1.8	9.6	L.5
T4-10b	....	1.6	8.6	L.6
T1-10f	....	0.0	4.4	L.6
T1-10c	....	....	0.0	L.7

corresponding to the lower values in table 2 indicate the interchange point to be near *li* rather than in the distal end of the short arm. The remaining two translocations with breaks in the short arm show the order to be *T - li - g - R*. Values for T8-10c are probably inflated because of the failure to recognize *li* plants in the presence of *g* resulting in a depression in the *+ li g +* non-crossover class and an inflation in the *+ + g +* class representing crossovers in regions 1 and 2. Similar difficulties were encountered with two other translocations T6-10a and T8-10b. Only the data from the non-golden plants are considered reliable and are presented in table 4. Though the numbers are small, translocation points of both are indicated to the left of *li*. The cytological positions of T3-10a, T6-10a, and T8-10b at 10L.12, 10L.19, and 10L.14 respectively, and the indication of the translocation points to the left of *li* in all three, make it probable that *li* is beyond .1 on the long arm.

A rough approximation of the serial order of the translocation points from left to right using the combined genetic and cytologic evidence is shown in table 5.

SUMMARY

Recombination values of the interchange point in 18 translocations involving chromosome 10 with *g* and *R* are summarized and compared with the cytologically determined positions. Four translocations appear to involve the short arm; and thirteen are probably on the long arm; and in one, some of the genetic data are inconsistent with cytological data.

Comparison of interchange positions on the long arm with *T-g* and *T-R* recombination values gave significant and highly significant correlation coefficients of -0.71 and $-.85$ respectively. Recombinations in the *g-R* region were homogeneous for all translocations in which *T-g* recombination exceeded two percent.

The gene *li* is on the long arm, probably at least 0.1 of the distance from the centromere.

The approximate order of the translocation points is indicated.

ACKNOWLEDGMENTS

The junior writer gratefully acknowledges the Gosney Fellowship of the California Institute of Technology, and the sabbatic leave from Purdue University which made possible his assistance in the summarization of these data.

LITERATURE CITED

ANDERSON, E. G., 1935 Chromosomal interchanges in maize. Genetics **20**: 70–83.

1938 Translocations in maize involving chromosome 9. Genetics **23**: 307–313.

1939 Translocations in maize involving chromosome 8. Genetics **24**: 385–390.

1941 Translocations in maize involving the short arm of chromosome 1. Genetics **26**: 452–459.

ANDERSON, E. G., and R. A. BRINK, 1940 Translocations in maize involving chromosome 3. Genetics **25**: 299–309.

ANDERSON, E. G., and L. F. RANDOLPH, 1945 Location of the centromeres on the linkage maps of maize. Genetics **30**: 518–526.

BURNHAM, C. R., 1949 Chromosome segregation in maize translocations in relation to crossing over in interstitial segments. Proc. Nat. Acad. Sci. **35:** 349–356.

1950 Chromosome segregation in translocations involving chromosome 6 in maize. Genetics **35:** 446–481.

BURNHAM, C. R., and J. L. CARTLEDGE, 1939 Linkage relations between smut resistance and semisterility in maize. J. Amer. Soc. Agron. **31:** 924–933.

CATCHESIDE, D. G., 1938 The bearing of the frequencies of X-ray induced interchanges in maize upon the mechanism of their induction. J. Genet. **36:** 321–328.

EMERSON, R. A., G. W. BEADLE and A. C. FRASER, 1935 A summary of linkage studies in maize. Cornell Univ. Agr. Exp. Sta. Mem. **180:** 1–83.

LONGLEY, A. E., 1950 Cytological analysis of translocations in corn chromosomes resulting from ionizing radiation of the test Able atomic bomb and X-rays and of translocations from other sources. *In* Report of Naval Research Section, Joint Task Force, ONE, on Biological aspects of Atomic Bomb Tests. Appendix No. 10, 1950. (Mimeograph)

MCCLINTOCK, G., and H. E. HILL, 1931 The cytological identification of the chromosome associated with the R-G linkage group in *Zea mays*. Genetics **16:** 175–190.

PATTERSON, E. B., 1952 Studies on crossing over in homozygous and heterozygous chromosome rearrangements in *Zea mays*. Ph.D. Thesis, California Institute of Technology, Pasadena: 1–111.

RHOADES, M. M., 1950 Meiosis in maize. J. Hered. **41:** 58–67.

RHOADES, V. H., 1935 The location of a gene for disease resistance in maize. Proc. Nat. Acad. Sci. **21:** 243–246.

RHOADES, M. M., and V. H. RHOADES, 1939 Genetic studies with factors in the tenth chromosome in maize. Genetics **24:** 302–314.

ROMAN, H., and A. S. ULLSTRUP, 1951 The use of A-B translocations to locate genes in maize. Agron. J. **43:** 450–454.

SABOE, L. C., and H. K. HAYES, 1941 Genetic studies of reactions to smut and of firing in maize by means of chromosomal translocations. J. Amer. Soc. Agron. **33:** 463–470.

STADLER, L. J., 1933 On the genetic nature of induced mutations in plants. II. A haploviable deficiency in maize. Mo. Agr. Exp. Sta. Res. Bul. **204:** 1–29.

GENE ACTION AND POSITION EFFECT IN DUPLICATIONS IN DROSOPHILA MELANOGASTER[1]

FRANK J. RATTY[2]
Department of Genetics, University of Utah, Salt Lake City, Utah

Received December 15, 1953

GENETIC material may be present in slight excess of the usual diploid content in *Drosophila melanogaster* without undue consequence to the individual. However, if the quantity of duplicated material is relatively large, then various abnormalities characteristic of hyperploidy may be observed; sterility may occur, and in extreme cases the condition may be lethal. Although these different effects may simply be the result of an upset in the normal genic balance, genes in duplicated regions may not function normally because of position effects. Especially subject to position effect would be genes in short duplications which have been inserted by translocation into heterochromatic regions. Indeed, various examples of such short insertional duplications of the white and other loci have been studied genetically by DEMEREC (1940) and cytologically by SUTTON (1940). In one case in particular, some twenty salivary chromosome bands including white (*w*, locus 1.5), roughest (*rst*, 1.7), facet (*fa*, 3.0), Notch (*N*, 3.0), and diminutive (*dm*, 4.6) loci have been inserted in inverted order into the heterochromatic region of the left arm of the third chromosome. Males and females with this insertion present as a duplication, Dp(1;3)$N^{264\text{-}58}$, are viable and fertile. However, rather extreme mottling of the first three visible loci is observed and diminutive is expressed as a recessive (see also description in BRIDGES and BREHME 1944; and GERSH 1952).

DEMEREC (1940) investigated the ability of this mottled duplication to compensate for, or "cover," the lethal effect of short deficiencies in the white-Notch region. In this paper, a duplication will be said to cover a lethal if any males with the lethal in combination with the duplication survive. In only one case of several studied ($N^{264\text{-}53}$, a translocation showing no cytological deficiency) was the duplication effective in covering the lethal. Even here the covered males were sterile. The conclusion seemed warranted that, ordinarily, the position effect on one or more genes in the duplicated region prevented them from functioning in a sufficiently normal manner to support life during embryonic development.

[1] This study was conducted at the University of Utah in partial fulfillment of the requirements for the degree of doctor of philosophy and was completed during the tenure of an Atomic Energy Predoctoral Fellowship.

[2] Present address: Department of Poultry Husbandry, University of California, Berkeley, California.

In the present study, three classes of lethals have been used in tests of the ability of Dp(1;3)$N^{264\text{-}58}$ and other similar duplications to cover lethal effects within various regions of their extents. These lethals consisted of *w*, *N*, and *w-N* deficiencies. It seemed important to determine if the same pattern of coverage observed in Dp(1;3)$N^{264\text{-}58}$ would be found in other mottled duplications of similar genetic length. Since the visible loci in Dp(1;3)$N^{264\text{-}58}$ are expressed in a mosaic fashion, it is also possible that genes affecting viability are similarly expressed. However, variegated position effects are not observed in euchromatic rearrangements. Therefore, the action of duplications inserted into euchromatin was compared with that of duplications inserted into heterochromatin. If a difference in gene action does exist, then mottled and non-mottled duplications of the same region should possess different capacities to cover the lethal effect of a given deficiency. In other words, the loss of normal function of the genes inserted into heterochromatin because of position effect should not occur if the genes were translocated to euchromatin.

Extensive coverage tests of five different duplications of the *w-N* region (including Dp $N^{264\text{-}58}$) have been carried out. From the results of these tests some conclusions have been drawn concerning the efficiency with which mottled and non-mottled duplications are capable of covering lethals within their extents.

MATERIALS AND METHODS

In order to determine the ability of the duplications to cover lethal effects, a series of cytologically known deficiencies, including $N^{264\text{-}58}$ from which Dp(1;3)$N^{264\text{-}58}$ was recovered, were obtained from DR. M. DEMEREC (see fig. 1) and tested with Dp (1;3)$N^{264\text{-}58}$ and other similar duplications. By observing which deficiencies were covered and which were not, a correlation could be made between the cytological length of a duplication and its genetic length in terms of the known gene loci it would cover. Then, the genetic extents of cytologically unknown deficiencies could be determined by coverage tests with the duplications. All five duplications have been tested extensively for their ability to cover deficiencies of the white, roughest, and split loci.

The duplications

Five short duplications each containing the white locus and each inserted into an autosome, have resulted from irradiation (fig. 1). Each is sufficiently short so that duplication males and females are both viable and fertile. A description of each follows:

1. Dp(1;3)$N^{264\text{-}58}$. *Duplication from chromosome 1 to chromosome 3 of the Notch locus* DEMEREC 38d. (DEMEREC 1940; SUTTON 1940) A section extending from salivary chromosome band 3B3-4 (BRIDGES' 1938 map) through band 3D5-6 is inserted in the heterochromatin of the left arm of the third chromosome. It contains the *w, rst, fa, spl* and *dm* loci. The *w, rst, spl* and *fa* phenotypes are all mottled because of their association with proximal heterochromatin, while the *dm* phenotype is not mottled and is expressed in

a recessive manner. This duplication resulted from the irradiation of a yellow (y) stock.

2. Dp(1;3)w^{49a7}. *Duplication from chromosome 1 to chromosome 3 of the white locus* LEFEVRE, 49a7. (DIS-23:59 and DIS-25:71) This duplication extends from band 3B1-2 through 3E1-2. It shows mottling for the *w, rst*

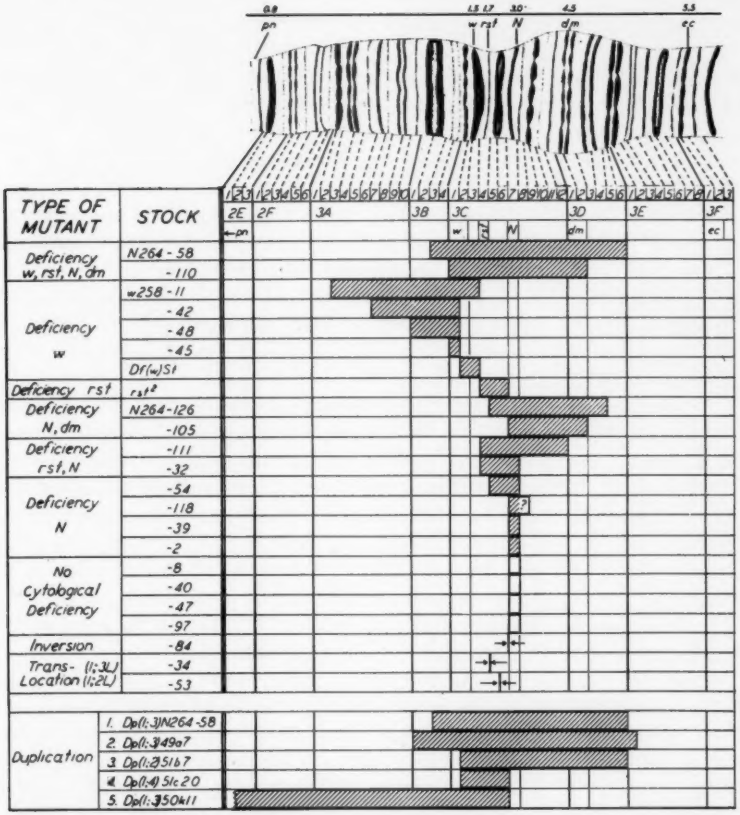

FIGURE 1.—A genetic and salivary map of the white-Notch region of the X-chromosome indicating the cytological extent of twenty-three deficiencies of this region as well as Dp(1;3)N^{264-58} and four other duplications of the white locus (after SLIZYNSKA 1938).

and *spl* loci while *dm* is recessive, and it resulted from the irradiation of a Canton-S$^+$ male. It is inserted in the heterochromatin of 3L and is in most respects similar to DpN^{264-58}.

3. Dp(1;2)w^{51b7}. *Duplication from chromosome 1 to chromosome 2 of the white locus* LEFEVRE, 51b7. (DIS-25:71 and DIS-26:66) This is a non-mottled duplication containing the *w, rst, spl, fa* and *dm* loci. It extends from

band 3C2 through 3D5-6 and is inserted in section 52F of the right arm of the second chromosome. It resulted from the irradiation of In(1)w^{m4} (inversion white-mottled-4) and therefore the euchromatic loci contained all normally lie to the right of 3C1. It shows good viability in normal males.

4. Dp(1;4)w^{51c20}. *Duplication from chromosome 1 to chromosome 4 of the white locus* LEFEVRE, 51c20. (DIS-25:71 and DIS-26:66) This is a mottled duplication containing *w* and *rst*. It is a section of the X-chromosome extending from 3C2 to and probably including 3C5-6 and is inserted in the heterochromatic region of chromosome 4. It also was produced by the irradiation of w^{m4}. Since the duplication covers Df(1)rst^2 but not *spl*, the right hand break point must be between 3C4 and 3C7.

5. Dp(1;3)w^{50k11}. *Duplication from chromosome 1 to chromosome 3 of the white locus* LEFEVRE, 50k11. (DIS-25:71, and DIS-26:66) This is a non-mottled duplication containing *pn* (prune), *w* and *rst*, but not *spl* or *fa*. It extends from band 1E3-4 through 3C6 and is inserted into section 89A of the right arm of the third chromosome. This duplication exhibits rather poor viability in normal males. It was recovered from a Notch stock resulting from irradiation of wild type Canton-S. Since cytologically, the duplication does not contain *N*, there was probably another fragment produced which included N^+, making three breaks in the X-chromosome.

For convenience, the following abbreviated symbols for these duplications will be used hereafter. This symbolization will denote: (1) whether or not the visible loci are mottled, and (2) the genetic extent of the duplication.

1. Dp^m(*w*-*N*)1. This symbolization indicates that the duplication, Dp(1;3)-$N^{264\text{-}58}$, is mottled and covers deficiencies in the region from white through Notch.

2. Dp^m(*w*-*N*)2. This duplication, Dp(1;3)w^{A9a7}, is mottled and also covers deficiencies in the region from white through Notch.

3. Dp^+(*w*-*dm*). This duplication, Dp(1;2)w^{51b7} is non-mottled and covers deficiencies in the region from white through diminutive.

4. Dp^m(*w*-*rst*). This duplication, Dp(1;4)w^{51c20}, is mottled and covers deficiencies in the region from white through roughest.

5. Dp^+(*pn*-*rst*). This duplication, Dp(1;3)w^{50k11}, is non-mottled and covers deficiencies in the region from prune through roughest.

The deficiencies

A variety of different types of lethals located in the *w*-*N* region were obtained from various sources. One particular white deficiency was produced by combining two similar but not identical inversions in a female, namely In(1)w^{m4} and In(1)rst^3 (inversion roughest-3). A single crossover produces a deficiency for salivary band 3C2-3 (PANSHIN 1941). Genetically, this crossover between w^{m4} and rst^3 results in a *w* deficiency which has been used as a standard for testing all suspected w^+ duplications. The chromosome carrying this white deficiency contains the left break of w^{m4} and the right break of rst^3. All five duplications of w^+ listed above cover this particular *w* de-

ficiency and give rise to viable and fertile Df(w) ; Dp(w^+) males. For convenience, this standard w deficiency will be referred to hereafter as Df(w)St. All duplication stocks are maintained as Df(w)St; Dp(w^+) ♂♂ × *y w f* double-X ♀♀.

A few deficiencies were obtained from an experiment conducted to obtain sex-linked lethal mutations. These deficiencies were produced by the irradiation of w^{48k24} and *y w sn*^{3} chromosomes and were balanced over Muller-5.

Very few sex-linked lethal mutations from the above experiment were found to be covered by the w^+ duplications. Therefore, Canton-S$^+$, w^{m4}, and *rst*^{3} males were irradiated to produce deficiencies specifically in the *w-N* region. The advantage of irradiating w^{m4} lies in the assurance that in any *w* deficiency produced, whatever euchromatic material in the normal gene order is lacking will be located just to the right of band 3C1. If such a *w* deficiency does not include Notch (located at 3C7), then the euchromatic deficiency necessarily is shorter than any of the five duplications and should be covered by all of them.

On the other hand, *w* deficiencies produced in *rst*^{3} chromosomes will lack euchromatic material which, in the normal gene order, lies just to the left of 3C4.

Ninety-one *w*, *N* and *w-N* deficiencies were produced from irradiations of Canton-S$^+$, w^{m4} and *rst*^{3} males. They were balanced over *y sc w*^{e} *spl* or *y*^{2} In(1)*sc*^{8} *f* In(1)dl-49 *v w*^{a} chromosomes. All of these deficiencies were tested for coverage with one or more of the duplications.

Finally, twenty-two, *w*, *N*, *w-N* and *rst* deficiencies of cytologically known extent were kindly provided by DR. M. DEMEREC for analysis with the duplications (see fig. 1). Three Notch deficiencies of unknown cytological extent were obtained from the irradiation of Abruptex (*Ax*, Dp 3C7).

Procedure for testing coverage of deficiencies by duplications

A uniform procedure was followed throughout in testing the capacity of each duplication to cover deficiencies located in the *w-N* region. Females which were heterozygous for the lethal and a non-lethal balancing inversion were mated to Df(w)St; Dp males. The proof of coverage consisted of the appearance of F_1 male progeny expressing the phenotype associated with the deficient chromosome carried heterozygously in the female parents. When the deficient chromosome being tested was *y*, then a *y*$^+$ Df(w)St; Dp male was used in order to detect non-disjunction, and vice versa. The correct identification of the covered male class was thus assured. Such covered males were then tested for fertility and, if fertile, were mated in order to demonstrate that they really carried the lethal in question.

With few exceptions at least three hundred, and frequently more, progeny were raised in each coverage test. In each case where coverage was possible, about one-third of all males counted should have been covered duplication-deficiency males, providing the lethal-duplication males and males with the balancer chromosome were equally viable. Although half the male zygotes

would contain the lethal chromosome, half of these would die since they lack the duplication which was heterozygous in the paternal male. Survival of the male zygotes containing the balancer chromosome would not depend on the duplication.

RESULTS

Coverage of deficiencies with duplications

Three of the duplications, $Dp^m(w\text{-}N)1$, $Dp^m(w\text{-}N)2$ and $Dp^+(w\text{-}dm)$, were able to cover many different cytologically known and unknown Notch mutants (see fig. 2). These duplications all include salivary band 3C7, deficiency of which produces Notch. The results of the coverage test with the cytologically known and unknown Notches together with other genetic evidence made it possible to derive certain conclusions regarding the limits of the cytologically unknown deficiencies.

Two additional duplications, $Dp^+(pn\text{-}rst)$ and $Dp^m(w\text{-}rst)$, contained the white but not the Notch locus (fig. 1). Since all five duplications contained the w^+ locus, they were all utilized in the coverage tests of the *w* deficiencies.

The coverage of Notches

The analysis of the cytologically known N (non-white) deficiencies. Fifteen Notch mutants were available which were known to be within the limits of $Dp^m(w\text{-}N)1$, which extends from 3B3 to 3D6. Eight of these were deficiencies lacking bands between 3C5 and 3D4, four were Notch mutants which had cytologically normal chromosomes, one was an inversion with the left break point between 3C6 and 3C7, and two were translocations. These Notches were also tested with $Dp^m(w\text{-}N)2$ and $Dp^+(w\text{-}dm)$. Figure 2 shows which of these deficiencies is covered by each of these three duplications, and when covered, the efficiency of the duplications in coverage in terms of the percentage of covered males among the total number of males examined. The Notches listed in figure 2 are presented in descending order of coverage by $Dp^m(w\text{-}N)1$. The coverage of the Notches in the two columns under the heading $Dp^m(w\text{-}N)2$ and $Dp^+(w\text{-}dm)$ should be compared with the values in the first column.

Figure 2 shows that nine of the cytologically known Notches were covered by $Dp^m(w\text{-}N)1$ and by $Dp^m(w\text{-}N)2$, whereas twelve were covered by $Dp^+(w\text{-}dm)$. This result indicates that all three duplications could restore a degree of normality to many of these Notch chromosomes such that they were no longer lethal, and the duplication-deficiency Notch male embryos were capable of development to the adult stage. This should not necessarily be taken to imply that all of these covered adult males were fully normal. If the coverage restored the normal physiological and genetic functions of the mutant chromosomes to a value equal to that of the balancer chromosome, then 33% of the male progeny should have been represented by the covered class. Percentages significantly greater than this value may represent occasional instances in which the covered lethal chromosome produced somewhat greater viability

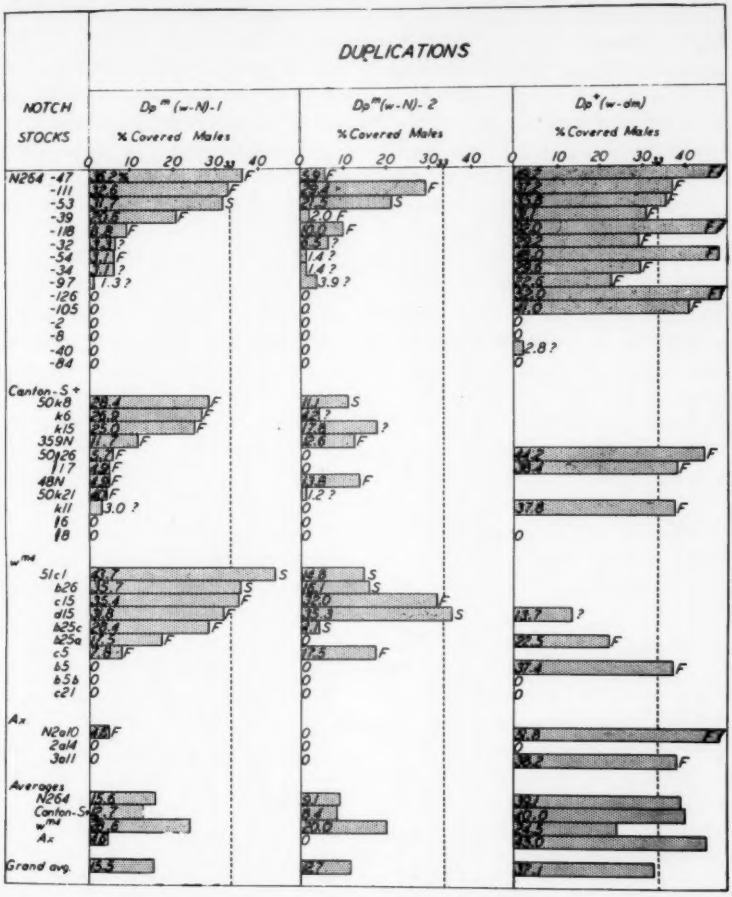

FIGURE 2.—The percentages of covered males containing Notch deficiencies of cytologically known and unknown extent which were produced in Canton-S^{+}, w^{m4}, and *Ax* chromosomes and which were tested for coverage by Dp^{m}(*w*-*N*)1, Dp^{m}(*w*-*N*)2 and Dp^{+}(*w*-*dm*) where 33% covered males were expected in each case. It is further indicated that F = fertile, S = sterile, ? = fertility unknown, and 0 = coverage was not possible.

than the balancer chromosome. The 33% level of coverage was realized in some of the tests, but many showed a relatively small percentage of coverage. Additional evidence that the covered males produced by each of the three duplications were not completely normal is the fact that covered males were not always fertile. For example, in the case of $N^{264\text{-}32}$ and $N^{264\text{-}53}$, the males covered by Dp^{m}(*w*-*N*)1 and Dp^{m}(*w*-*N*)2 were sterile, but those covered by Dp^{+}(*w*-*dm*) were fertile.

Each duplication possessed a characteristic ability to cover each *N*. One duplication may produce a large number of covered males, whereas another duplication, tested for coverage with the same deficiency, produces few. $Dp^{+}(w\text{-}dm)$ regularly produced the largest percentage of covered males with each tested cytologically known *N*. $Dp^{m}(w\text{-}N)1$ covered the same deficiencies less efficiently than $Dp^{+}(w\text{-}dm)$ but better than $Dp^{m}(w\text{-}N)2$. The average ability of each duplication to cover all of the cytologically known Notches was: $Dp^{+}(w\text{-}dm)$, 39.1%; $Dp^{m}(w\text{-}N)1$, 15.6%; and $Dp^{m}(w\text{-}N)2$, 9.1%. The cases showing no coverage by any of the duplications were not included in these averages. This could be done legitimately because the inability of the duplications to cover a known short deficiency was not generally a defect of the duplications, but rather was a characteristic of the deficient chromosome resulting from a second independent sex-linked lethal. This interpretation was tested with respect to several cytologically known Notch mutants of short extent: $N^{264\text{-}2}$, $N^{264\text{-}8}$ and $N^{264\text{-}84}$. Since these Notches were all derived from irradiation experiments and have since been kept in balanced stocks, they might well be accompanied by independently produced sex-linked lethals. This expectation was clearly demonstrated in the case of $N^{264\text{-}8}$. Crossover analysis of $N^{264\text{-}8}$ showed that a second lethal, located between *ec* and *ct*, was present. After the removal of the second lethal, $N^{264\text{-}8}$ was easily covered. Similar demonstrations were made in several other cases. Coverage tests also indicated the presence of apparently independent sex-linked sterility factors within the limits of one or more of the duplications.

It may be noted that in some cases a deficiency having no independent lethal was not covered by a duplication that appears cytologically to contain all the missing material. Thus, $N^{264\text{-}126}$ and $N^{264\text{-}105}$ (deficiencies extending from *N* through *dm*) were covered by $Dp^{+}(w\text{-}dm)$ but not by $Dp^{m}(w\text{-}N)1$ or $Dp^{m}(w\text{-}N)2$. Cytologically, $Dp^{m}(w\text{-}N)1$ and $Dp^{m}(w\text{-}N)2$ extend at least two and four bands, respectively, farther to the right than do the two Notch deficiencies; and the left break points of the deficiencies are well within their limits. However, these duplications not only show mottling for the visible loci within the *w-N* region but are also genetically recessive for *dm*.

The coverage of cytologically unknown Notches. When a random sample of cytologically unknown Notches was tested for coverage by $Dp^{m}(w\text{-}N)1$, $Dp^{m}(w\text{-}N)2$ and $Dp^{+}(w\text{-}dm)$, it was found that the coverage of these Notches produced by irradiation of Canton-S^{+}, w^{m4} and *Ax* was quite similar to the coverage of the cytologically known Notches (fig. 2). In each case tested where $Dp^{m}(w\text{-}N)1$ covered one of the unknown Notches, $Dp^{+}(w\text{-}dm)$ also did. However, $Dp^{m}(w\text{-}N)2$ failed to cover three of the Canton-S^{+} and one of the w^{m4} Notch deficiencies which $Dp^{m}(w\text{-}N)1$ covered. As with the cytologically known Notches, $Dp^{m}(w\text{-}N)1$, though cytologically shorter, proved to have better covering power than $Dp^{m}(w\text{-}N)2$. The average coverage by $Dp^{m}(w\text{-}N)1$ of the randomly produced Notches was 15.3% as compared with 15.6% for its coverage of the cytologically known Notches. $Dp^{m}(w\text{-}N)2$ produced 14.3% covered males from the random Notches as compared with 9.1% for the known Notches. $Dp^{+}(w\text{-}dm)$ produced the highest proportion

of covered males: 36.5% for the unknown Notches and 39.1% for the known Notches. Of the twenty-four experimentally produced Notches which were tested, five were not covered by any duplication. It might be expected that of the Notches tested, about 13% would have independent lethals produced at the time of irradiation with 5000 r. The remaining uncoverable Notches could be the result of deficiencies having a cytological extent greater than that of any of the duplications.

The coverage of white-Notch deficiencies

An analysis of the coverage of *w-N* deficiencies showed that each duplication possessed the ability to cover fairly large as well as small deficiencies (see fig. 3). However, no cytologically known *w-N* deficiencies were available which were within the cytological limits of $Dp^m(w\text{-}N)1$, $Dp^m(w\text{-}N)2$

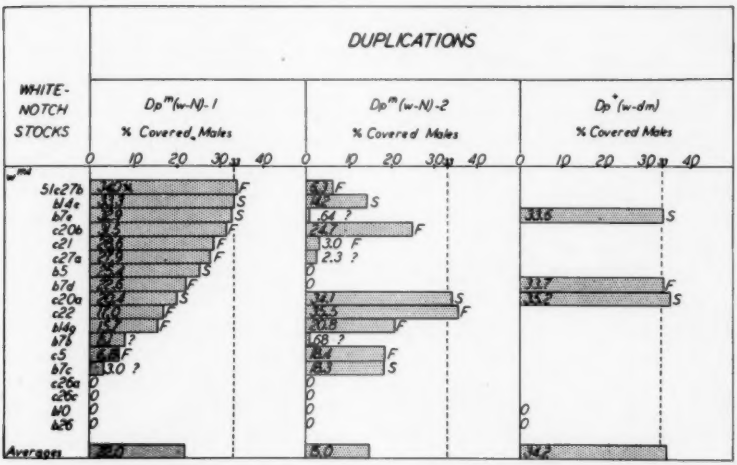

FIGURE 3.—The percentages of covered males containing white-Notch deficiencies which were produced in w^{m4} chromosomes and which were tested for coverage by $Dp^m(w\text{-}N)1$, $Dp^m(w\text{-}N)2$ and $Dp^+(w\text{-}dm)$ where 33% covered males were expected in each case. It is further indicated that F = fertile, S = sterile, ? = fertility unknown, and 0 = coverage was not possible.

and $Dp^+(w\text{-}dm)$. The shortest *w-N* deficiency of cytologically known extent was $N^{264\text{-}58}$, from which $Dp^m(w\text{-}N)1$ was recovered. The latter would not cover the former, although the duplication included all the missing bands of the deficiency. This indicated that the genetic extent of $Dp^m(w\text{-}N)1$ was shorter than its cytological extent.

Eighteen *w-N* deficiencies of unknown cytological extent, but derived from irradiation of w^{m4}, were tested for coverage by $Dp^m(w\text{-}N)1$, $Dp^m(w\text{-}N)2$ and $Dp^+(w\text{-}dm)$. Four of the eighteen were not covered by any of the duplications. Thus 22% of the w^{m4} *w-N* deficiencies were not covered as compared with 21% of the unknown Notches which were not covered. The behavior of

two or three of the non-coverable white-Notches could be accounted for by an extra lethal while the others perhaps possessed deficiencies of cytological extent greater than that of the duplications. As was the case with the random Notches, each *w-N* deficiency that was covered by $Dp^m(w\text{-}N)1$ was also covered by $Dp^+(w\text{-}dm)$. $Dp^m(w\text{-}N)2$ failed to cover two of the deficiencies which were covered by the other two duplications, again suggesting that genetically it is the shortest of the three duplications. $Dp^+(w\text{-}dm)$ produced the largest proportion of covered *w-N* males (34.2%), as compared with $Dp^m(w\text{-}N)1$ (22.0%) and $Dp^m(w\text{-}N)2$ (15.0%).

Most of the *w-N* deficiencies were also tested with $Dp^+(pn\text{-}rst)$. None were covered, as would be expected, since $Dp^+(pn\text{-}rst)$ does not cover split.

The coverage of w deficiencies

The analysis of the cytologically known w deficiencies. Coverage tests were conducted on five cytologically known *w* deficiencies: $w^{258\text{-}11}$, $w^{258\text{-}42}$, $w^{258\text{-}45}$, $w^{258\text{-}48}$ and Df(*w*)St (see fig. 4). Figure 1 shows that only $Dp^+(pn\text{-}rst)$ is of such an extent that it would be expected to cover all of these deficiencies. Actual tests showed that $Dp^+(pn\text{-}rst)$ did cover all of the deficiencies except $w^{258\text{-}45}$. Deficiency $w^{258\text{-}45}$ should have been covered by all of the duplications, except $Dp^+(w\text{-}dm)$ and $Dp^m(w\text{-}rst)$, since it is only deficient for 3C1 (salivary analysis by SUTTON). Cross over analysis of $w^{258\text{-}45}$ indicated that this chromosome contained an independent sex-linked lethal factor in addition to the deficiency for 3C1.

Except for $Dp^+(pn\text{-}rst)$, the other duplications, $Dp^+(w\text{-}dm)$, $Dp^m(w\text{-}N)1$, $Dp^m(w\text{-}N)2$ and $Dp^m(w\text{-}rst)$, were not expected to and did not cover $w^{258\text{-}42}$ and $w^{258\text{-}48}$. This was because cytologically these deficiencies extend beyond the left limit of the duplication. All five of the duplications covered Df(*w*)St.

The coverage of cytologically unknown w deficiencies. White deficiencies which were experimentally produced in Canton-S^+, w^{m4} and rst^3 chromosomes were tested for coverage. Since the w^{m4} inversion separates 3C1 from 3C2-3, the latter band must represent the left extent of *w* deficiencies as they would appear in a normal chromosome. Of the ten w^{m4} white deficiencies tested, none were Notch but most of them appeared to be deficient for *rst*. Therefore, the right break of these deficiencies occurred between 3C4 and 3C7. Hence, all of the *w* deficiencies which were produced in w^{m4} chromosomes and did not contain independent lethals should have been covered by all of the duplications. This prediction was substantiated by the fact that nine of the ten w^{m4} *w* deficiencies tested could be covered. $Dp^+(pn\text{-}rst)$ produced 31.2% covered males, $Dp^+(w\text{-}dm)$ 41.3%, $Dp^m(w\text{-}N)1$ 30.5%, $Dp^m(w\text{-}N)2$ 44.0% and $Dp^m(w\text{-}rst)$ 43.0% (see fig. 4).

Since the *w* deficiencies produced in rst^3 chromosomes must all extend to the left of *w* (band 3C2-3), it was anticipated that very few of them would be covered by $Dp^+(w\text{-}dm)$, $Dp^m(w\text{-}N)1$, $Dp^m(w\text{-}N)2$ and $Dp^m(w\text{-}rst)$. Of the sixteen rst^3 *w* deficiencies tested, only two were covered by these duplications while eleven were covered by $Dp^+(pn\text{-}rst)$.

The *w* deficiencies which were produced in Canton-S^+ chromosomes dif-

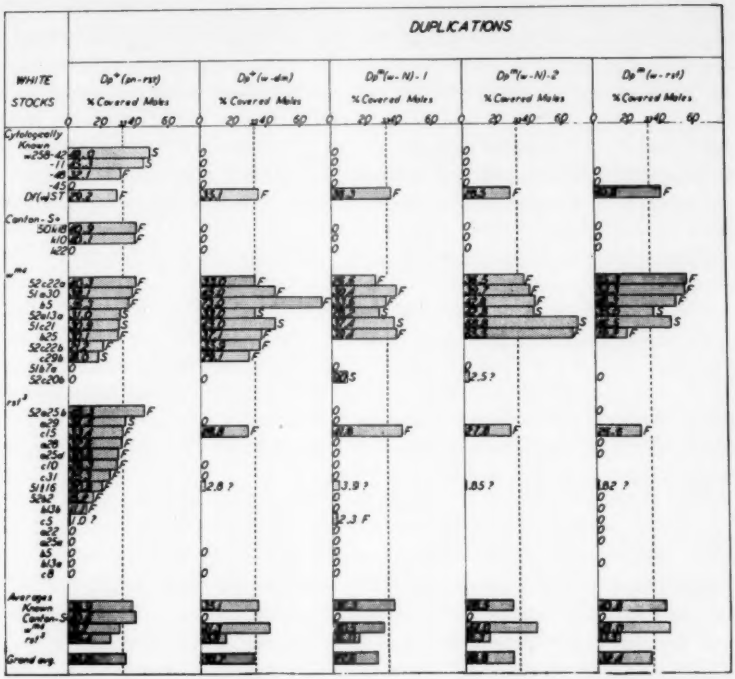

FIGURE 4.—The percentages of covered males containing white deficiencies of cytologically known and unknown extent which were produced in Canton-S$^+$, w^{m4}, and *rst*^{3} chromosomes and which were tested for coverage by Dp$^+$(*pn-rst*), Dp$^+$(*w-dm*), Dp^m(*w-N*)1, Dp^m(*w-N*)2 and Dp^m(*w-rst*) where 33% covered males were expected in each case. It is further indicated that F = fertile, S = sterile, ? = fertility unknown, and 0 = coverage was not possible.

fered from those produced in *rst*^{3} only in that they could have included additional bands between 3C3 and 3C7. As expected, Dp$^+$(*pn-rst*) covered a greater proportion of these deficiencies than the other duplications because it extends a greater distance to the left.

DISCUSSION

The duplications used in this study were of two types: mottled (MULLER 1930; SCHULTZ 1936) and non-mottled. The term mottled refers to a position effect producing a variegated phenotypic expression of the genes located in the duplications which are associated, in our cases, with heterochromatin of one of the autosomes. The heterochromatin of all of the autosomes seems to be capable of invoking this type of expression. Since gene action in duplications was tested by coverage of lethals in the X-chromosome, it is of interest to compare the efficiency of the mottled and non-mottled duplications in covering similar lethal effects. Because of the mottling of the visible loci, it might

be predicted that there would also be position effects on other genes in the mottled duplications, genes whose function is concerned with viability but which do not possess a visible expression. If this prediction were true, then the mottled duplications should not cover deficiencies because of suppression of vital functions by the heterochromatin (DEMEREC 1940). That this is not always true of the mottled duplications has been adequately demonstrated by the present study. Mottled duplications, as well as non-mottled duplications, are capable of covering lethal mutants, although not always with equal efficiency.

Mottled rearrangements present two expressions of position effect. One is a position effect on the visible expression of loci and the other is concerned with lethality. Some rearrangements produce a variegated position effect but do not affect the viability of the individual. For example, the w^{m4} inversion produces mottled eyes with little or no effect on the viability of the flies. However, some aberrations give rise to a position effect whose expression is lethality in addition to its effect on the visible loci concerned. This type of lethal position effect is seen in $N^{264\text{-}6}$, $N^{264\text{-}9}$ and $N^{264\text{-}10}$. SCHULTZ (cited in BRIDGES and BREHME 1944) has shown that $N^{264\text{-}6}$ is a translocation with the break in the X-chromosome following 3C9. Heterozygous females are Notch, with variegation of the genes in the w-N region. The XY males usually die or are sterile; however, XYY males live, are sterile, and show no variegation. In this situation, the position effect spreads from the break point at 3C9 to produce the lethal Notch condition at 3C7. Proof that the expression of this N is associated with position effect is shown by the fact that the addition of an extra Y-chromosome, which reduces mottling, also eliminates the lethality and produces a viable, though sterile, male. Both $N^{264\text{-}9}$ and $N^{264\text{-}10}$, also translocations, react similarly to extra Y-chromosomes.

In the present study, all of the Notch mutations were male-lethal, presumably resulting from deficiencies of 3C7. However, the expression of the Notch phenotype in some of the N mutants produced in w^{m4} appeared to be correlated with the degree of mottling. For example, some stocks showed variability in the degree of eye pigmentation, and the Notch phenotype was expressed only in those individuals which had the lightest mottled eye color. In these cases, the addition of extra Y-chromosomes might be expected to cover the lethal position effect. However, the mottled duplications themselves do not show such a position effect on lethality, except where a vital portion of the embryo was not included in the normal part of the variegation pattern.

With the exception of those Notch mutants that resulted from position effects, the importance of extra Y-chromosomes on coverage in the present experiment was, at best, only negligible. That Y-chromosome effects were not generally involved is evidenced by the following observations. (1) There was no significant difference ($\chi^2 = .61$, $.2 < P < .5$) in the number of non-disjunctional offspring among the progeny of Notch mutants with only 5% covered males as compared with those tested with the same duplication having 30% or more. (2) A plot of the percent covered males against the proportion

of exceptional males per total females of crosses involving a given Notch mutant indicates a complete disparity between the number of non-disjunctional male offspring in cases of high and low coverage. (3) Cytological observations of the chromosomes in brain smears of duplication-deficiency males substantiate the above observations inasmuch as they do not demonstrate the presence of extra Y-chromosomes. Even in the event that Y-chromosomes should play some minor role in coverage, it seems improbable that their presence would be sufficient to account for the differences in efficiency of mottled duplications which were tested with the same group of lethals.

The coverage of Notches. Both genetic and cytological evidence has shown that mottled as well as non-mottled duplications are capable of covering lethal effects within their limits, thereby producing viable males which otherwise would have died. In a few cases, it was found that duplications which should have covered deficiencies within their limits did not do so (figs. 1 and 2). Non-coverage of such deficiencies appears to be a function of the deficiency chromosome which in each case tested contained an independent lethal rather than reflecting an incapacity of the duplications to cover deficiencies within their extents. In cases where the second lethal was eliminated, coverage has been made possible. The genetically active length of the duplication has always determined whether a lethal condition can be covered. When coverage is possible with one of the shorter duplications, the same deficiency will always be covered by a longer one. This has been demonstrated by the coverage of the Notch mutants by $Dp^m(w\text{-}N)1$ and $Dp^+(w\text{-}dm)$. Every *N* which is covered by the former is also covered by the latter (fig. 2).

However, there is some discrepancy between the cytological and genetic evidence regarding the extent of the insertion in the case of the mottled duplications. The genetic evidence has indicated that $Dp^m(w\text{-}N)1$ and $Dp^m(w\text{-}N)2$ are not capable of covering lethal effects along their entire cytological extent. For example, they will not cover $N^{264\text{-}105}$, $N^{264\text{-}110}$ or $N^{264\text{-}126}$. The reason for this is not clear since $Dp^m(w\text{-}N)1$ and $Dp^m(w\text{-}N)2$ are known to extend cytologically as far as 3D6 which is well beyond the right limits of these three *N* deficiencies (BRIDGES and BREHME 1944). $Dp^m(w\text{-}N)1$ and $Dp^m(w\text{-}N)2$ may have lethal position effects associated with their right breaks which spread to affect nearby loci in the same way that mottling can spread to visible loci removed from the point of breakage. Moreover, $Dp^m(w\text{-}N)1$ shows a recessive rather than a variegated phenotypic expression of *dm* located at 3D1-2, as also does $Dp^m(w\text{-}N)2$. This could be due to (1) mutation or deletion of dm^+ in the duplication, or (2) position effect on the locus. Since no bristles of normal length are ever observed in *dm* males covered by $Dp^m(w\text{-}N)1$ or $Dp^m(w\text{-}N)2$, it must be assumed that if a lethal position effect is the explanation, it must be of a stable type and spreads for only a limited distance. This situation is not encountered in the other duplications, however, and the lack of coverage by $Dp^m(w\text{-}N)1$ and $Dp^m(w\text{-}N)2$ may be an exception rather than the rule.

Even though a *N* mutation may be covered and produce a viable male, the

Notch phenotype is usually not evidenced. It might be anticipated that the Notch phenotype would be expressed in a Notch-duplication male, since the genotype of such a male is quite analogous to that existing in a N/N^+ female. That this prediction is not fulfilled indicates that the prerequisite for the expression of Notch is a function of genic balance in addition to the loss of salivary band 3C7. The Notch phenotype is observed in N/N^+ and in N/N; Dp(N^+) females, but not in N/N^+; Dp(N^+) females nor in N; Dp(N^+) males. Therefore, the expression of the Notch phenotype results from the relationship which occurs when only one N^+ locus, regardless of its location, is present in a female genotype; however, one N^+ locus in a male produces wild-type wings.

Although a duplication will usually cover any lethal effect within its limits, the coverage of the same deficiency by different duplications indicates that each duplication possesses a characteristic ability to cover a deficiency. The N mutants were thoroughly tested with Dp^m(w-N)1, Dp^m(w-N)2 and Dp$^+$(w-dm), and it was found that the non-mottled duplication covered the Notches more efficiently than did the mottled ones. This relationship perhaps results from position effects on the genes in the mottled duplications, which were unable to compensate for the genetic and physiological functions of the deleted loci as well as genes in Dp$^+$(w-dm) which was translocated to euchromatin.

If the action of heterochromatin on variegation is the same throughout its entirety, it would be predicted that similar duplications translocated to the heterochromatin of the same chromosome would exhibit similar coverage. This situation was not realized, however, as evidenced by the different ability of Dp^m(w-N)1 and Dp^m(w-N)2 to cover Notches. These duplications have nearly identical genetic lengths and cytological break points. They are both located in the proximal heterochromatin of the left arm of the third chromosome. Nonetheless, Dp^m(w-N)1 consistently produced a higher proportion of covered N males than did Dp^m(w-N)2 (see figs. 2 and 3). Therefore, the proportion of covered males from the N tests depends upon: (1) the Notch deficiency being entirely within the limits of the duplication, (2) whether the duplication is mottled or not, and (3) the specific extent and location of the duplication. This indicates that the Notch mutants are genetic alterations of such a nature that the presence of the mottled duplications will not necessarily completely compensate for the deficiency. However, Dp$^+$(w-dm) is capable of fully restoring the normal function to most of the N mutants. This is demonstrated by the fact that it produced on the average 37.1% (33% expected) covered Notch males, while Dp^m(w-N)1 and Dp^m(w-N)2 produced only 15.5% and 12.7%, respectively, where the same proportion was expected.

The coverage of w deficiencies. The coverage of the white deficiencies indicates that the nature of the w locus is different from that of the N locus. Coverage tests showed that the mottled duplications cover w deficiencies as well as the nonmottled ones (fig. 4). The average percentage of covered white-lethal males produced by each duplication was: Dp^m(w-N)1, 27.6%;

$Dp^m(w\text{-}N)2$, 28.8%; $Dp^m(w\text{-}rst)$, 32.4%; $Dp^+(w\text{-}dm)$, 30.7%; and $Dp^+(pn\text{-}rst)$, 34.0%. Among the mottled duplications, there is no correlation between the ability to cover a *N* deficiency and a *w* deficiency. For example, $Dp^m(w\text{-}N)1$ covers *N* mutations more efficiently than $Dp^m(w\text{-}N)2$, but the two are similar in their ability to cover *w* deficiencies. Apparently, loss of the w^+ locus or some loci very close to it with no visible expression gives rise to a less fundamental upset in development than loss of the N^+ locus does. Alternatively, the effect of mottling on the *w* region is less effective as far as viability is concerned than on the *N* locus.

The coverage of w-N deficiencies. The coverage of the *w-N* deficiencies by $Dp^m(w\text{-}N)1$, $Dp^m(w\text{-}N)2$ and $Dp^+(w\text{-}dm)$ is quite similar to that observed for the *N* deficiencies. It shows that the mottling associated with $Dp^m(w\text{-}N)1$ and $Dp^m(w\text{-}N)2$ is of such a nature that they cannot easily cover *w-N* deficiencies. This is indicated by the differential ability of the mottled and non-mottled duplications to cover *w-N* deficiencies. The proportion of covered *w-N* males approximated that of the covered *N* males which were produced by the two mottled duplications, $Dp^m(w\text{-}N)1$ and $Dp^m(w\text{-}N)2$. For example, $Dp^m(w\text{-}N)1$ produced 15.5% and 22.0% covered *N* and *w-N* males, while $Dp^m(w\text{-}N)2$ produced 12.7% and 15.0%, respectively. Neither of the mottled duplications produced an average percentage of *N* or *w-N* males as great as the lowest figure observed in their corresponding *w* deficiency coverage. On the other hand, $Dp^+(w\text{-}dm)$ produced 37.1% covered *N* males, 34.2% covered *w-N* males and 30.7% covered *w* deficiency males. This demonstrates again that the non-mottled duplication is capable of restoring a degree of normalcy such that the expected proportion of covered males (33%) is usually approximated regardless of the particular locus or combination of loci it is covering. Therefore, although the criteria for coverage or lack of coverage appears to be a characteristic of the deficiency chromosome, the efficiency with which a duplication will cover a deficiency depends upon whether the duplication is mottled or not.

The chromosome from which $Dp^m(w\text{-}N)1$ was produced ($N^{264\text{-}58}$) was tested for coverage by $Dp^m(w\text{-}N)1$, $Dp^m(w\text{-}N)2$ and $Dp^+(w\text{-}dm)$. It was not covered by any of the duplications, even Duplication $Dp^m(w\text{-}N)1$ which cytologically has the same extent as the deficiency (SUTTON 1940). The inability of $Dp^m(w\text{-}N)1$ to cover $N^{264\text{-}58}$ might be due to a lethal position effect at one or both break points or to a deficiency of genetically active material which was not cytologically detectable. However, none of the duplications studied covered the deficiency from which it was derived, although most of the former appear to be cytologically equivalent to the latter. This might indicate that X-ray produced alterations, such as the duplications used in this study, are always associated with the loss of some genetically active material, perhaps located in an interbandular area, necessary for the viability of the individual. Alternatively, in the neighborhood of break points there is regularly produced a lethal position effect which may spread to a greater or lesser extent.

SUMMARY

1. Five mottled and non-mottled duplications produced by irradiation were tested for their ability to cover deficiencies in the *w-N* region of the X-chromosome.

2. All of the duplications covered lethal effects within their cytological limits except $Dp^m(w\text{-}N)1$ and $Dp^m(w\text{-}N)2$ which would not compensate for deficiencies of the *dm* region.

3. The non-mottled duplications covered *N* and *w-N* deficiencies more efficiently than did the mottled ones; by contrast there were no significant differences in the ability of the mottled and non-mottled duplications to cover *w* deficiencies.

4. The differences observed in the coverage data of *N* and *w* deficiencies may be interpreted as indicating that the *N* locus is concerned with more fundamental developmental activities than the *w* locus, or alternatively, the variegated position effect on the *w* region is less effective as far as viability is concerned than it is on or near the *N* locus.

ACKNOWLEDGMENTS

The author wishes to acknowledge with thanks the assistance and guidance of DR. GEORGE LEFEVRE, JR. during the course of this study, also that of DR. E. R. DEMPSTER and DR. ELDON J. GARDNER who have read the manuscript and DR. W. W. NEWBY who, in addition, helped with the preparation of the figures. The author is also indebted to DR. H. J. MULLER for the special stocks provided and especially to DR. M. DEMEREC for the series of deficiencies involving the white-Notch region, without which the study would have been far less complete. Acknowledgment is also extended DEAN JOHN Z. BOWERS, DR. HENRY PLENK, DR. JOHN KARR and DR. MARVIN VAN DILLA of the school of Medicine, University of Utah, for permission to use and assistance in operating the X-ray machines employed in this study.

LITERATURE CITED

BRIDGES, C. B., and K. BREHME, 1944 The mutants of *Drosophila melanogaster*. Carnegie Inst. Wash. Publ. 552.

DEMEREC, M., 1940 The genetic behavior of euchromatic segments inserted into heterochromatin. Genetics **25**: 618–627.

GERSH, E. S., 1952 Pigmentation in a mottled white eye due to position effect in *Drosophila melanogaster*. Genetics **37**: 322–338.

MULLER, H. J., 1930 Radiation and genetics. Amer. Nat. **64**: 220–251.

PANSHIN, I. B., 1941 Cytogenetic analysis of the homology of genes in reversed linear repeats. Comp. rend. (Doklady) Acad. Sci. USSR **30**: 57–60 (in English).

SCHULTZ, J., 1936 Variegation in *Drosophila* and "inert" chromosome regions. Proc. Nat. Acad. Sci. **22**: 27–33.

SLIZYNSKA, H., 1938 Salivary chromosome analysis of the white-facet region of *Drosophila melanogaster*. Genetics **23**: 290–299.

SUTTON, E., 1940 Structure of salivary gland chromosomes of *Drosophila melanogaster* in exchanges between euchromatin and heterochromatin. Genetics **25**: 534–540.

GENETIC DRIFT IN IRRADIATED EXPERIMENTAL POPULATIONS OF DROSOPHILA MELANOGASTER [1]

TIMOTHY PROUT

Department of Zoology, Columbia University, New York City [2]

Received December 20, 1953

THE investigation reported in the present paper is of the effects of genetic drift in three experimental populations of *Drosophila melanogaster,* two of which received treatments with gamma rays. Although there has been considerable theoretical discussion of genetic drift, few experimental studies of this phenomenon have been carried out. This paucity of experimental verification exists in spite of the fact that WRIGHT (1931 and other papers) has described several concrete situations where the effects of genetic drift could be studied and measured experimentally.

One of these situations may be found in a single population in which there are a number of loci with a pair of alleles at each locus. If all these alleles are subject to the same evolutionary forces (mutation, selection, migration, etc.) then the variance of allele frequencies may be taken as a measure of genetic drift. In other words, genetic drift will tend to cause the alleles at some loci to be represented more times than the average and some less than the average.

It is this model which will be used in the present investigation of the effects of genetic drift on the behavior of recessive lethal alleles at various loci on the second chromosome of *Drosophila melanogaster.*

This approach is essentially the same as that used by DOBZHANSKY and WRIGHT (1941), WRIGHT, DOBZHANSKY and HOVANITZ (1942) and IVES (1945). In their studies the assumption was made that a Drosophila chromosome carries a certain number of loci which mutate to recessive lethal alleles at finite rates. Each locus may then be represented by either a lethal or a nonlethal allele. Samples of chromosomes were taken from the natural populations studied, and analyzed to determine which of them carried recessive lethal alleles. Strains carrying the lethal bearing chromosomes were then crossed among themselves to determine which chromosomes carried allelic lethals. This investigation of allelism showed the number of times different loci were represented in the sample by lethal alleles. In turn this information allowed evaluation of the amount of genetic drift which occurred in the populations studied.

The authors cited above carried out their studies on the third chromosome in certain natural populations of *D. pseudoobscura* and on the second chromosome in populations of *D. melanogaster.*

The investigation reported in the present article is concerned with studies

[1] Submitted in partial fulfillment of the requirements for the degree of Doctor of Philosophy in the Faculty of Pure Science, Columbia University, New York City, 1953.

[2] Present Address: Division of Life Sciences, University of California, Riverside, California.

on the allelism of lethals in the second chromosomes of artificial laboratory populations kept by DR. BRUCE WALLACE and his associates.

THE EXPERIMENTAL POPULATIONS

Three of a series of populations raised and studied by DR. BRUCE WALLACE (1949, 1950, 1951, 1952) have been used. The important characteristics of these populations are summarized in table 1. Two of the populations (Nos. 3 and 6) contained more flies than the third (No. 5). Populations Nos. 5 and 6 received continuous treatment with gamma rays, while the remaining population, No. 3, served as control. The populations are kept in lucite cages described by WALLACE (1949). The population size in the cages is controlled by regulating the food supply. The radiation source is 500 mg of radium placed at the center of curvature of the semicircular cages. The population Nos. 3, 5 and 6 of table 1 designate the same populations as Nos. 3, 5 and 6 of WALLACE's papers. Egg samples are taken every two weeks or multiples of this time which is the presumed generation time (WALLACE 1950a). The flies

TABLE 1

Description of populations showing amount of radiation in roentgens per hour (r/hr) and per generation (r/generation), lethal mutation rates for the second chromosome, and estimated number of individuals (size).

Population	r/hr	r/generation	Mutation rate %	Size
3	0	0	.45	$\sim 10^4$
6	5.1	2000	5.0	$\sim 10^4$
5	5.1	2000	5.0	$\sim 10^3$

raised from these eggs are used in a series of crosses designed to reveal the presence or absence of lethals in the second chromosome (WALLACE 1951a).

Figure 1 shows the history of these three populations with respect to the frequencies of recessive lethal chromosomes. The data up to generation 34 for Population No. 3 and generation 17 for Population Nos. 5 and 6 have been reported and discussed by WALLACE (1951), who has also estimated the mutation rates in the populations shown in table 1.

It can be seen that, as expected, the populations receiving radiation (Nos. 5 and 6) accumulated much greater frequencies of lethals than the unirradiated population No. 3. The frequency of lethal chromosomes in the two irradiated populations apparently have approached equilibria in the vicinity of 80% to 85% lethal chromosomes. The unirradiated population No. 3 appears to be reaching an equilibrium at a much lower frequency, namely around 30% lethal chromosomes.

EXPERIMENTAL DETERMINATION OF ALLELISM

As stated above, DR. WALLACE and his collaborators have taken periodic samples from the experimental populations, and have determined the frequencies of lethal bearing second chromosomes in these samples. The lethals extracted from some of these samples (taken at times indicated in figure 1 by

arrows) were kindly given by DR. WALLACE to the writer for determination of the allelism of these lethals.

In order to study the allelism in a given sample of lethal bearing second chromosomes, all the possible heterozygotes between these lethal bearing chromosomes are made. If the same lethal gene is carried on two or more chromosomes in the sample, then a lethal zygote will be formed every time these chromosomes meet. If a lethal gene is represented only once in the sample, then the chromosome carrying it will form viable heterozygotes with all other

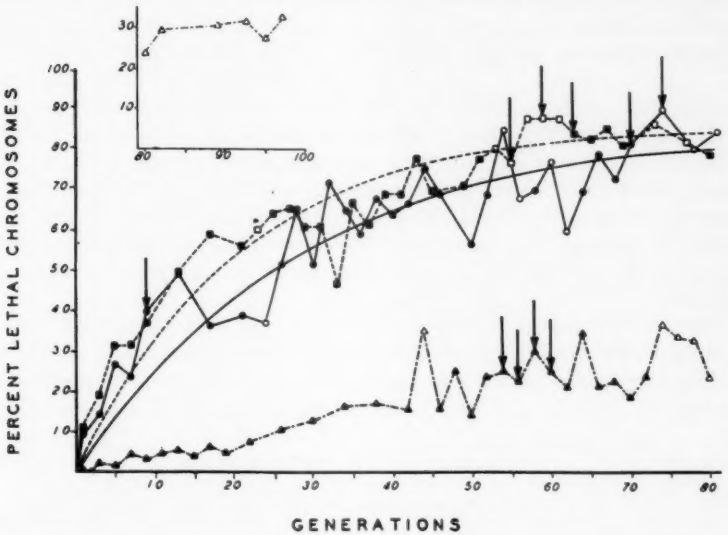

FIGURE 1.—Percent of second chromosomes found to be homozygous lethal in the three populations over a period of time: Triangles connected by long and short dashes, ▲– – – – –△, represent population 3; squares connected by regular dashes, ■– – – – –□, represent population 6; and circles connected by solid lines, ●————○, represent population 5. Solid points represent samples of greater than 60 chromosomes; open points represent samples of less than 60 or equal to 60. The inserted graph is the continuation of population 3.

lethal chromosomes in the sample. By making all possible crosses among strains with lethal chromosomes, the number of times various lethal genes are represented can be determined. Further, a total measure of allelism can be made by computing the total frequency of lethal zygotes formed. This measure is called "the frequency of allelism."

The experimental procedure was as follows: The extraction process leaves each lethal chromosome balanced with the "Curly-Lobe" marker chromosome. The latter chromosome carries two long inversions, one in each arm, to prevent crossing over with the lethal bearing chromosome, and also contains the two dominant markers, Curly Wing (*Cy*) and Lobe eye (L^4). The chro-

mosome is lethal in double dose so that balanced lethal cultures can be maintained indefinitely. Only one cross is necessary in order to determine whether a lethal bearing chromosome carries a lethal gene allelic to a lethal gene on another lethal bearing chromosome. Virgin females from one balanced lethal culture are crossed with males from another, and absence of wild type flies among the progeny of this cross indicates that the lethals involved are alleles.

For a series of lethal chromosomes all possible cross combinations were made, viz., for K lethal chromosomes there are $K(K-1)/2$ crosses. The crosses were made in " creamers "; two virgin females and at least three males were used for each cross. Reciprocal crosses were not made. The distinction between allelic and non-allelic cultures was always unequivocal.

THE RESULTS OF ALLELISM TESTS

Table 2 shows the results of the allelism crosses for the three populations. The time the sample was taken (in generations from the start of the population), the number of chromosomes sampled, and the number of crosses made are shown. Also shown are the numbers of lethal genes represented once, twice, three times, etc., among the lethal chromosomes sampled. Finally, the total number of cases of allelism observed and the frequency of allelism are shown.

In the columns headed " singles " are shown the numbers of chromosomes carrying lethal genes which were found to be allelic to no other lethals on the other chromosomes of the sample. In parentheses are shown the estimated numbers of lethal genes represented only once among all chromosomes of the

TABLE 2

These data concern the allelism in the three populations, showing the generations from which lethal chromosomes were sampled, the number of chromosomes in the sample, the number of crosses made among these chromosomes, and the number of loci found to be represented once or more times in the sample.

Population	Generation	Number of chromosomes	Number of crosses	1	2	3	4	5	>5	Total alleles	% allelism
3	Within 54,56,58,60	65	581	59	3	0	0	0	0	3	.516
3	Between 54,56,58,60	65	1499	53	6	0	0	0	0	6	.403
3	Total	65	2080	47(57)	9	0	0	0	0	9	.433
6	Within 55,59,63	99	1801	62	14	3	0	0	0	23	1.28
6	Between 55,59;59,63	99	1394	70	10	0	0	0	0	10	.72
6	Total	99	3195	62(179)	24	3	0	0	0	33	1.03
5	Within, 9	47	1081	33(42)	4	2	0	0	0	10	.93
5	Within, 70	35	595	8	4	2	0	0	1(13)	87	14.6
5	Within, 70[#]	22	231	13(30)	3	1	0	0	0	6	2.6
5	Within, 74[†]	47	1081	11	4	3	0	1	1(14)	114	10.5
5	Within, 74[†]	33	528	15(58)	4	2	1	0	0	16	3.0
5	Within, 70[#],74[†]	55	759	28(88)	7	3	1	0	0	22	2.9

[#]Discounting chromosomes carrying lethal present 13 times.
[†]Discounting chromosomes carrying lethal present 14 times.

sample. The difference between the number of single chromosomes and number of single genes is due to the fact that a lethal chromosome may carry several lethal genes. The number of lethal genes represented singly can be computed by making the assumption that the lethal genes are distributed on the chromosomes according to the terms of a Poisson series. It can be seen from the table that the ratio of lethal genes to lethal chromosomes is much higher in populations with a high frequency of lethal chromosomes (Nos. 5 and 6) than in Population No. 3 where this frequency is low.

The allelism for Population 3 was determined from small samples taken from four different generations: 16 chromosomes from generation 54, 11 chromosomes from generation 56, 27 chromosomes from generation 58, and 11 chromosomes from generation 60.

It might be expected that the frequency of allelism between generations would be lower than that within generations. This effect is expected because the particular lethal alleles present at a given time in a population are not necessarily identical with those present at another time. Although the data show a difference in the expected direction, the difference is not significant. The total frequency of allelism is 0.433%. This value may be considered characteristic of this population during the period, generation 54 to 60.

For Population 6, 36 chromosomes were used from generation 55, 17 from generation 59, and 46 from generation 63. Again the frequency of allelism within generations was higher than the allelism between generations but not significantly so. The total frequency of allelism, 1.03%, can therefore be said to characterize Population 6 for the period between generations 55 and 63. This value of 1.03% is considerably higher than the frequency of allelism obtained for the unirradiated Population 3.

It should be pointed out that because the ratio of lethal genes to lethal chromosomes is much higher in Population 6 than in Population 3, a higher frequency of allelism in the former population is expected due to this factor alone. This will be discussed later.

Three samples were taken from the small irradiated Population 5, 47 chromosomes from generation 9, 35 chromosomes from generation 70, and 47 from generation 74. The most outstanding result of the crosses is the presence in the sample taken in generation 70 of a lethal allele which has reached an extremely high frequency. This lethal has persisted in the population for at least four generations at a frequency of 30.1% in generation 70 and 26.7% in generation 74. In generation 70, where one lethal was represented 13 times, no other lethals were present more than three times. In generation 74, where the same lethal was present 14 times, no other lethal was present more than five times. These facts make it unlikely that genetic drift was responsible for the high frequency of this gene. It is more probable that this lethal produces heterosis on the genetic background and under the environmental conditions obtaining in the experimental population. Favorable selection in the heterozygous condition would then lead to accumulation of heterozygotes carrying the lethal. For the purposes of studying genetic drift, therefore, only the

allelism due to lethals other than the presumably heterotic one should be considered. Thus the allelism of chromosomes not carrying the high frequency lethal were computed in each case, generations 70 to 74, as shown in table 2. The combined allelism for the period, generations 70 to 74 was also computed (2.9%).

It can be seen that the small population in general had a higher frequency of allelism than the two large populations. In generation 9 the small population had a higher frequency of allelism than Population 3 had even in generations 54 to 60, although in this case the frequency of lethal chromosomes was higher in generation 9 of the small population (39.4%) than in Population 3 (25.5%). In generation 9 the small population had a frequency of allelism equal to that attained by Population 6 only in generation 55. And yet Population 6 had a much higher frequency of lethal chromosomes at that time (82.5%) than that possessed by the small population in generation 9. During the period, generations 70 to 74 the allelism in the small population (discounting the heterotic lethal) was significantly higher ($\chi_1^2 = 8.47$) than that in Population 6, although in this case the frequencies of lethal chromosomes were similar.

ANALYSIS OF THE ACCUMULATION CURVES

The hypothesis that genetic drift is operating to a greater degree in the small population than in the large ones permits two inferences to be drawn with relation to the accumulation curves of lethal chromosomes (fig. 1). First, the frequency of lethal chromosomes should undergo greater fluctuations from sample to sample in the small population. Second, the frequency of lethal chromosomes should be lower in a small than in a large population (WRIGHT 1937). The small population will only be compared with Population 6, since Population 3 was not subject to the same conditions of radiation.

By inspection of figure 1 it can be seen that the frequency of lethal chromosomes from time to time fluctuates more in the small Population 5 than in the comparable large Population 6. In order to obtain a quantitative measure of the fluctuations in these two populations, correlations were computed between successive values of Q, the frequency of lethal chromosomes. This correlation would be perfect, $r = 1$, in both populations if there were no sampling errors, no genetic drift, and if Q_{n-1}, the lethal frequency in generation $n - 1$, were actually a linear function of Q_n, the lethal frequency in generation n. All three of these factors tend to reduce the correlation. The components due to lack of linearity and to sampling errors should reduce the correlation to an equal extent in both populations. On the other hand, the drift component should act differentially, reducing this correlation in the small population more than in the large population. The correlation coefficients between successive values of Q are shown in table 3. $r = 0.948$ for the large Population 6 and $r = 0.890$ for the small Population 5. The difference between these correlation coefficients is not significant, but the difference is in the expected direction.

In computing the above correlation coefficients the assumption was made

TABLE 3

Least square estimates of $\hat{Q}$, the equilibrium frequency of lethal chromosomes, and k, the rate constant. r is the correlation coefficient of successive frequencies of lethal chromosomes.

Population	$\hat{Q}$	k	r
6	.865	.047	.948
5	.857	.035	.890

that Q_{n-1} is a linear function of Q_n. This is, of course, incorrect. In fact the actual sequence equation is known (DOBZHANSKY and WRIGHT 1941, p. 40). From this linear approximation, however, it is possible to obtain a function which describes the accumulation curve of lethal chromosomes. This function is

$$Q = \hat{Q}(1 - e^{-KG}), \tag{1}$$

where

Q = the frequency of lethal chromosomes after G generations,
$\hat{Q}$ = the equilibrium frequency of lethal chromosomes,
G = the number of generations elapsed,
K = a rate constant.

Equation (1) is a curve which at least geometrically probably does not differ to any great extent from the actual theoretical accumulation curve. For this reason curves of this form were fitted by least squares to the observed points of Populations 2 and 3. Estimates of the parameters Q and K so obtained are shown in table 3. The curves are reproduced in figure 1. As already stated it can be seen that the observed points of the small population appear to fluctuate more about their corresponding fitted curve than the points of Population 2 about their curve. The contribution to these fluctuations in both curves by sampling errors may be judged by inspection of the points, where in the small population solid circles represent samples with more than 60 chromosomes, and open circles represent samples with 60 or fewer chromosomes; solid squares and open squares represent samples with more than 60 and 60 or fewer respectively in the large population. The smallest sample sizes were 19 chromosomes of which there were only two, generation 23 of Population 6 and generation 24 of Population 5. Most of the samples contained 80 chromosomes, while there were a few samples with more than 200, which were taken from both populations in the first 20 generations. Aside from a few exceptions which can be seen from the graph, the samples taken at approximately the same time from the two populations were very similar and often identical in size.

Although Population 3 was not considered in the above analysis, its accumulation curve is also shown in figure 1 (designated by open and solid triangles). The sudden increase in fluctuations after generation 40 is due simply to a substantial decrease in the size of the samples taken after generation 40.

That the small population possesses a lower frequency of lethal chromosomes than Population 6 is not immediately evident from figure 1. The expectation

that the frequency of lethals in the small population should be low applies strictly only to the final equilibrium, while most if not all of the data from the two populations come from preequilibrium generations. But it may be expected that the small population, since it should eventually reach a lower equilibrium, should show lower frequencies of lethal chromosomes than the large population during comparable preequilibrium generations. This expectation is borne out. Comparing the frequencies of lethals observed in the two populations at similar times, only in 8 out of 32 comparisons were the lethal frequencies in the small population higher than those observed in the large population at the same time, and these 8 were contributed mostly by early generations. If the expected frequencies of lethals after a given number of generations were alike for the two populations, then half the time (or 16 times in this case) the small population should show a higher frequency than the large. The observed number deviates significantly from this expectation of equality.

The above argument is further strengthened when the occurrence of the high frequency lethal is considered. The contribution of this lethal to the total frequency of lethal chromosomes is large. But as has already been pointed out, the high frequency of this lethal probably cannot be explained by genetic drift. It therefore is more meaningful to compare the frequency of lethal chromosomes in the large population with the frequency in the small population due to alleles at loci other than that of the heterotic lethal.

The frequencies of such chromosomes were therefore computed from the data and it was found that for generations 70 and 74 respectively 73.0% and 85.9% of all chromosomes carried a lethal allele at at least one locus other than that producing the heterotic lethal. The total frequencies of lethal chromosomes including the heterotic lethal were 81.1% for generation 70 and 89.7% for generation 74. The difference (including vs. excluding the heterotic lethal) in generation 70 is considerable, but not so great in generation 74. Evidently this heterotic lethal was contributing considerably to the observed total frequencies of lethal chromosomes for a number of generations prior to its detection in generation 70. Therefore it can be concluded that the frequencies of chromosomes carrying lethals at other loci in recent generations (say 60 to 80) has been somewhat lower than the total frequencies observed. This fact then is additional evidence that the small population is approaching a lower equilibrium than the large population as would be expected as a result of genetic drift.

ESTIMATION OF POPULATION PARAMETERS

The observed frequency of alleles among lethals will now be used to estimate certain population parameters, namely, variance of the gene frequencies, the effective population size, and the average selection against heterozygotes for the lethals. WRIGHT has worked out the necessary mathematical relationships (DOBZHANSKY and WRIGHT 1941; WRIGHT, DOBZHANSKY and HOVANITZ 1942) which will be used in the following analysis.

The primary data. The primary information available is as follows:

Q = The frequency of chromosomes which carry at least one recessive lethal gene. These frequencies are shown in table 4.

P = The frequency of allelism between lethal bearing chromosomes taken at random from a population. The values of P have already been given in table 2 and are shown again in table 4.

V = The mutation rate which produces lethal chromosomes, i.e., the proportion of chromosomes per generation which acquire at least one lethal by mutation at any locus. The values shown in table 1 have been determined by WALLACE (1951a) for the irradiated (Nos. 6 and 5) and the unirradiated (No. 3) populations.

p_ϵ = The frequency of allelism of a random pair of independently arising lethal genes. This value was determined by IVES (1945) and WALLACE (1950b). The average of their two values is 0.0025, as shown in table 4, indicated by " Ind."

TABLE 4

Estimates of population parameters; p, the probability of allelism of a random pair of lethal genes, σ^2 the variance of gene frequencies, and N, the effective population size. C is the number of crosses made in determining P, the chromosome allelism.

Population	Generation	Q	P	C	p	$\sigma^2 \times 10^3$	N
3	54–60	.26	.0043	2080	.0032	.015	
6	55–63	.82	.0103	3195	.0023	−.152	
3 and 6					.0025	0	∞
5	9	.39	.0093	1081	.0078	.332	
5	70	.73	.026	231	.0205	7.72	288
5	74	.86	.030	528	.0197	16.62	279
5	70–74	.81	.029	759	.0212	12.59	256
Ind.					.0025		

Derived constants. The following properties of recessive lethals can be evaluated from the primary data listed above:

n = The number of loci on the second chromosome which regularly mutate to recessive lethal alleles.

$\bar{q}$ = The mean frequency of lethal alleles in the population per lethal producing locus.

σ^2 = The variance of lethal allele frequencies per lethal producing locus about the mean, $\bar{q}$.

p = The probability of allelism of a random pair of lethal genes (as opposed to the allelism of a random pair of lethal bearing chromosomes, P).

$\bar{v}$ = The mean mutation rate from non-lethal to lethal alleles per lethal producing locus.

P_ϵ = The probability of allelism of a random pair of lethal bearing chromosomes where the two chromosomes acquire their contained lethal genes independently.

$\overline{S}$ = The mean selection coefficient of heterozygotes between lethal and non-lethal bearing chromosomes.

$\overline{s}$ = The mean selection coefficient of heterozygotes between lethal genes and their non-lethal alleles.

Of ultimate interest are the two evolutionary parameters $\overline{s}$ and N, but it will be not only of interest but necessary to first examine the values of the remaining properties listed above for the three populations under consideration.

The variance of gene frequencies. WRIGHT (DOBZHANSKY and WRIGHT 1941) showed that the frequency of allelism of a random pair of lethal genes is a function of, among other things, the variance of the frequencies of lethal alleles from locus to locus.

$$\sigma^2 = \frac{\left(p - \frac{1}{n}\right)(n\overline{q})^2}{n} \tag{2}$$

This equation (2) for the variance of lethal gene frequencies has three unknowns, p, n, and the quantity $(n\overline{q})$ (the mean number of lethal genes per chromosome). The primary information will now be used to obtain independent estimates of these three unknowns.

First, it has already been indicated that there is a difference between the frequency of allelism of lethal bearing chromosomes and the allelism of random pairs of lethal genes. WRIGHT has given the following correction for a population of infinite size:

$$P_\infty = P_\infty\left(\frac{Q}{-\ln(1-Q)}\right)^2 \tag{3}$$

Equation (3) assumes that the number of lethal alleles carried by one chromosome of a zygote is independent of the number carried by the other. This condition would be satisfied by chromosomes coming from effectively infinite populations, from different populations, or by newly arisen lethal chromosomes. In finite populations, however, WRIGHT points out that in an appreciable number of zygotes the number of lethal alleles on one chromosome will be the same as the number on the other through common descent. The correction for finite populations he then gives as

$$p = p_* + (P - P_*) \tag{4}$$

It follows that p, the allelism of lethal genes, can be estimated from P, the allelism of lethal chromosomes, from Q, the frequency of such chromosomes, and from p_*, the probability of allelism of independently arising lethal genes. Thus a comparison can be made between the allelism in the three populations without introducing the complicating factor of the number of genes per chromosome.

For the three populations under study, it makes little difference whether the correction for finite population size (eq. 4) is used or equation (3) is applied directly. The allelism of lethal genes for the two large populations

(Nos. 3 and 6) is very close to the independent allelism figure, whereas the small Population 5 for all generations sampled showed the allelism always to be greater than the independent value. Equation (3) may be applied to the two large populations and equation (4) to the small population. The results so obtained are shown in table 4.

It can be seen that the gene allelisms, p, of the two large populations are very similar although the chromosome allelisms are quite different. The weighted mean of these two values of p is 0.0025 (designated "3 and 6" in table 4), which value is in agreement with the allelism of independently arising lethal genes, and in fact happens to be identical with it. The frequencies of allelism of lethal genes in the small population, however, are all larger than this value of p = 0.0025 for the large populations. As early as generation 9 the value of p = 0.0078 was obtained. The value of chi square testing the difference between p = 0.0025 and p = 0.0078 is $\chi_1^2 = 2.99$, which falls between the 0.10 and 0.05 levels of significance. During the period, generations 70 to 74 of the small population, the value of p = 0.0212 was obtained. Comparing this value with the large population value of 0.0025 gives a chi square of $\chi_1^2 = 7.95$, the probability of which is between 0.01 and 0.001. It should be mentioned here that these chi square values were computed on the basis of large sample theory, which is not strictly applicable here.

The two remaining unknowns in the formula for the variance (eq. 2), n and $(n\bar{q})$, must now be estimated. The number of lethal producing loci (WALLACE 1950b) can be obtained from the allelism of independently arising lethal genes by the relationship,

$$p_\infty = \frac{1}{n}\left(1 - \frac{\sigma_v^2}{\bar{v}^2}\right), \tag{5}$$

where,

σ_v^2 = variance in mutation rates;

If the assumption is made that variance of mutation rate is zero, or at least much smaller than the square of its mean, then

$$n = \frac{1}{p_\infty}. \tag{6}$$

Using the figure $p_\infty = 0.0025$ (table 4), the number of lethal producing loci on the second chromosome comes out to be n = 400 (WALLACE 1950b).

For an estimate of $n\bar{q}$, the mean number of lethal genes per chromosome, it is again assumed that the lethal genes have a Poisson distribution on the chromosomes, with a mean $n\bar{q}$, then

$$n\bar{q} = -\ln(1 - Q). \tag{7}$$

The results of applying this relationship to the data are shown in table 5. Also shown in table 5 are estimates of $\bar{q}$, which, of course, were obtained by dividing $(n\bar{q})$ by 400 (this estimate of $\bar{q}$ will be used later).

Estimates of the variance of the lethal gene frequencies can now be made

substituting the values obtained for n, ($n\bar{q}$), and p into equation (2). The values of the variance so obtained are shown under "$\sigma^2 \times 10^5$" in table 4. It should be pointed out that equation (2) estimates the total variance of gene frequencies due not only to genetic drift but also to variability in mutation rates and to variability in semidominance from locus to locus. This variance may then be considered as the sum of drift, selection and mutational components. In order to estimate n, and from the value of n to estimate the variance of gene frequency, it was necessary to assume that the variance of mutation rates is negligible. If this assumption is granted, the results shown in table 4 may be interpreted to mean that there is no variance in the large populations due either to drift or to selection. In the small population, however, a variance of gene frequencies was found as early as generation 9, and this variance increased to a value of $\sigma^2 = 1.7 \times 10^{-4}$ by generation 74. The simplest conclusion is that this variance in the small population is mainly due to the drift component.

TABLE 5

Estimates of $n\bar{q}$, the mean number of lethal genes per chromosome and $\bar{q}$, the mean frequency of lethal alleles per locus in the population.

Population	Generation	Q	$n\bar{q}$	$\bar{q} \times 10^3$
3	54–60	.26	.3011	.753
6	55–63	.82	1.715	4.29
5	9	.39	.4943	1.24
5	70	.73	1.309	3.27
5	74	.86	1.966	4.92
5	70–74	.81	1.661	4.15

This conclusion may also be reached by considering the data in another way. Using equation (4) it is possible to calculate the chromosome allelism expected in these populations assuming they were of infinite size.

This was done and it was found that in the case of the small population the observed allelism was significantly greater than that which would be expected on this assumption of infinite size ($\chi_1^2 = 7.57$ which falls between the .01 and .001 level of significance).

Effective population size. In a finite population, even with random mating and randomly varying family size, a variance of gene frequencies is expected to develop. This will occur because of the sampling errors involved in the choice of a finite number of gametes from the gene pool of one generation to produce the zygotes of the next. This random drift variance can be further increased by mating of relatives, or by increased variation of family sizes (that is, where a large number of individuals leave no offspring, or few individuals have a large number of offspring, or both). If one or both of these factors are in operation, then it is said that the effective population size is smaller than its absolute size. The effective size, therefore, should be an inverse function of the variance of gene frequencies. Wright has constructed such a function for the case of lethal genes:

$$\sigma^2 = \frac{\bar{q}(1 - \bar{q})(1 - \bar{v}/\bar{q})^2}{2N(1 - (1 - \bar{v}/\bar{q})^2) - (1 - \bar{v}/\bar{q})^2} \tag{8}$$

Solving for N, the effective population size,

$$N = \frac{\left(\frac{\bar{q}(1 - \bar{q})}{\sigma^2} - 1\right)(1 - \bar{v}/\bar{q})^2}{2(1 - (1 - \bar{v}/\bar{q})^2)}, \tag{9}$$

where $\bar{v}$, the mean mutation rate per locus, can be estimated by

$$\bar{v} = \frac{n\bar{v}}{n} = \frac{-\ln(1 - v)}{n} \cong \frac{V}{n} \tag{10}$$

For the irradiated populations equation (10) gives $\bar{v} = 1.25 \times 10^{-4}$ and for the unirradiated population, $\bar{v} = 1.13 \times 10^{-5}$. $\bar{q}$, the mean frequency per lethal gene, has already been estimated as shown in table 5. Equations (8) and (9) assume that equilibrium has been reached and that the variance of gene frequencies is due entirely to genetic drift. Estimates of the effective population sizes can then be made using equation (9).

Since no variance is found in the two large populations, these populations behave effectively as infinite ones, insofar as the detectable variance of lethal gene frequencies is concerned. This conclusion is evident since the allelism of lethal genes in these two populations is very close to the independent allelism value (table 4).

In the case of the small population, equilibrium clearly has not become established in generation 9 (fig.1), but the equilibrium condition was apparently approached in generations 70 and 74. Applying equation (9) to these two generations of the small population, two estimates of the effective population size, N = 288 and N = 279, are obtained. Also for the period, generations 70 to 74, the combined estimate is N = 256 (table 4). (This value, N = 256, does not fall between the values of N calculated for the two generations separately because rather than computing the average N for these two generations, the primary data were combined first and the resulting figures substituted into equation 9.) Because of the accumulated errors involved in making these estimates, the most that can be concluded is that the effective size of the small population is probably smaller than its absolute size. The latter was estimated to be around 1000 individuals (table 1).

Selection against heterozygotes. Heterozygotes carrying lethals have been shown by several authors to be, on the average, less viable than lethal free zygotes. CORDEIRO (1952), STERN (1952) and others (cf. CORDEIRO 1952, bibliography) measured the viability of heterozygotes directly. DOBZHANSKY, WRIGHT and HOVANITZ (1941, 1942), and PROUT (1952) applied an indirect method, based on the comparison of the rates of production and of elimination of the lethals in the populations. A method of the latter kind will be used for the data now under consideration.

The estimate of the average selection coefficient of heterozygotes for lethal chromosomes may be obtained from the primary data directly, by the relationship,

$$\bar{S} = \frac{V - PQ^2(1 + V)}{Q(1 + 2V) - 2PQ^2(1 + V)} \tag{11}$$

In table 6 in the column headed "$\bar{S}$" are shown the selection coefficients calculated in this way.

In order to compare populations with different frequencies of lethal chromosomes, it is desirable to get an estimate of the average selection against heterozygotes for lethal genes rather than lethal chromosomes. Assuming that a heterozygote where the lethal chromosome carries K lethal genes has adaptive value $(1 - K\bar{s})$, where $\bar{s}$ is the average selection coefficient of heterozygotes for lethal genes, the correction is,

$$\bar{s} = \bar{S}\frac{Q}{-\ln(1 - Q)}. \tag{12}$$

The values of $\bar{s}$ so calculated are shown in the rightmost column of table 6.

The values of $\bar{s}$ arrived at are of the same order of magnitude as those obtained for the effects of lethals in various natural populations of Drosophila by the authors cited above.

TABLE 6

$\bar{S}$ and $\bar{s}$ are the mean selection coefficients of heterozygotes for lethal chromosomes and lethal genes respectively.

Population	Generation	$\bar{S}$	$\bar{s}$
3	54–60	.016	.014
6	55–63	.047	.022
5	70	.043	.024
5	74	.027	.012
5	70–74	.036	.017

DISCUSSION

The orders of magnitude of three population parameters have been estimated for the three populations under study. These parameters are N, the effective population size, $\bar{s}$, the selection against lethals in heterozygous condition, and $\bar{v}$, the mutation rate per locus. The values obtained were N = 256 for the small population, and N is effectively infinite for the large populations; the average value of $\bar{s} = 0.018$ was characteristic of all three populations, and $\bar{v} = 1.25 \times 10^{-4}$ for the irradiated and $\bar{v} = 1.13 \times 10^{-5}$ for the unirradiated population. Under the assumption of equal evolutionary pressures on all loci, these three parameters completely define the distribution of lethal gene frequencies. In a sense, the populations examined have thus been completely described. It has been pointed out, however, that the errors involved in making these

estimates are large, and a more precise determination of the parameters must await further investigation.

On the other hand the data have provided strong evidence of the operation of genetic drift in at least one of the populations (No. 5). The comparison of the allelisms (p) or variances (σ^2) of the small and large populations, the apparently greater fluctuations of the accumulation curve of the small population, and the indication that the small population is approaching a lower equilibrium, are all factors pointing to the operation of genetic drift.

The study of lethal producing loci, then, has served as an effective method for detecting genetic drift. It is doubtful, however, that drifting lethal frequencies themselves can have any great effect on the evolutionary history of a population. It is obvious, however, that if there is drift variance in lethal frequencies there must be drift variance of the frequencies of less deleterious alleles. In fact selection is operating more severely against drift variance of lethal alleles than it is against the variance of less deleterious alleles. In the small population then there must be an even greater non-adaptive variance of the alleles which are expected to be of greater evolutionary significance than lethal alleles.

At this stage in the experimental investigation of genetic drift it is not of first importance that the material used for detecting drift be of evolutionary significance itself.

The phenomenon of genetic drift is the subject of much debate. The issue of this debate is not whether in theory genetic drift occurs. The laws of probability demand that in Mendelian populations a random component of the variance of gene frequencies must occur. Rather the question is whether this random component of variance has been large enough in an important number of natural populations in the past so that the resulting non-adaptive exploration of gene frequencies has provided the primary step in subsequent evolutionary transformations. In accordance with the prevailing theories of experimental evolution, evidence bearing on this question may be obtained from contemporary natural and experimental populations. Methods, therefore, must be devised and tested for measuring genetic drift; and these measurements should be made on a large number and variety of populations.

The present work then is another test of a method of measuring genetic drift, devised by DOBZHANSKY and WRIGHT. It also is submitted as another of the still small number of populations in which genetic drift has been measured.

SUMMARY

Second chromosomes carrying recessive lethal genes were extracted from laboratory populations of *D. melanogaster* by DR. BRUCE WALLACE and his collaborators. The frequency of allelism among these lethal bearing chromosomes was determined by the present author by means of a series of appropriate crosses.

Three populations were studied. One was a large population ($\sim$ 10,000

individuals) subject to chronic radiation; one was a small population ($\sim$ 1000 individuals) also irradiated; and one was a large population receiving no treatment.

The results of the tests of allelism showed the small population to have a significantly higher frequency of allelism than the two large populations.

Analysis of the curves of accumulation of lethal bearing chromosomes show that (a) the small population tends to reach a lower equilibrium frequency of lethals than the comparable large population, and (b) the frequencies of lethal chromosomes undergoes greater fluctuation in the small than in the comparable large population.

These results, from the allelism tests and the accumulation curve analyses furnish evidence that genetic drift operates in at least the small population.

Three population parameters are estimated according to methods devised by WRIGHT (DOBZHANSKY and WRIGHT 1941; WRIGHT, DOBZHANSKY and HOVANITZ 1942). These parameters are N, the effective population size; $\bar{s}$, the mean selection coefficient of heterozygotes for lethal genes; and $\bar{v}$, the mean mutation rate per locus.

The results of these estimates are as follows:

Population	N	$\bar{s}$	$\bar{v}$
Large, no radiation	∞	0.014	1.13×10^{-3}
Large, irradiated	∞	0.022	1.25×10^{-4}
Small, irradiated	256	0.017	1.25×10^{-4}

These estimates indicate that (a) the small population may have a genetically effective size smaller than its absolute size of $\sim$ 1000 individuals; and that the large population of size $\sim$ 10,000 cannot be distinguished from effectively infinite populations by this method; (b) the selection against heterozygotes is affected neither by population size nor by the irradiation. In fact the values of $\bar{s}$ obtained are of the same order of magnitude as those found in several wild populations of several species of Drosophila.

ACKNOWLEDGMENTS

The author wishes to express his deepest appreciation to PROFESSOR THEODOSIUS DOBZHANSKY for his continual guidance and encouragement throughout the course of this investigation and in the writing of the manuscript. PROFESSOR HOWARD LEVENE is also to be thanked for mathematical and statistical advice as well as for his many invaluable suggestions of a more general nature. The author is also indebted to DR. BRUCE WALLACE and his collaborators who not only supplied all the material for this study, but who also made all their data available to the writer. This study was carried out during the tenure of a United State Atomic Energy Commission predoctoral fellowship.

LITERATURE CITED

CORDEIRO, A., 1952 Experiments on the effects in heterozygous condition of second chromosomes from natural populations of Drosophila willistoni. Proc. Nat. Acad. Sci. 38: 471–478.

DOBZHANSKY, TH., and S. WRIGHT, 1941 Genetics of natural populations. V. Relation between mutation rates and accumulation of lethals in populations of Drosophila pseudoobscura. Genetics **26**: 23–51.

1943 Genetics of natural populations. X. Dispersion rates in Drosophila pseudoobscura. Genetics **28**: 304–340.

IVES, P. T., 1945 The genetic structure of American populations of Drosophila melanogaster. Genetics **30**: 167–196.

PROUT, T., 1952 Selection against heterozygotes for autosomal lethals in natural populations of Drosophila willistoni. Proc. Nat. Acad. Sci. **38**: 478–481.

STERN, C., 1952 The viability of heterozygotes for lethals. Genetics **37**: 413–449.

WALLACE, B., 1949 A new population cage. Dros. Inf. Serv. **23**: 108–109.

1950a Autosomal lethals in experimental populations of Drosophila melanogaster. Evol. **4**: 172–174.

1950b Allelism of second chromosome lethals in Drosophila melanogaster. Proc. Nat. Acad. Sci. **36**: 654–657.

1951a Genetic changes in populations under irradiation. Am. Nat. **85**: 209–222.

1951b Genetic changes within populations after X-irradiation. Genetics **36**: 612–628.

1952a A genetic analysis of the adaptive values of populations. Proc. Nat. Acad. Sci. **38**: 706–715.

1952b The estimation of adaptive values of experimental populations. Evol. **6**: 333–341.

WRIGHT, S., 1931 Evolution in mendelian populations. Genetics **16**: 98–159.

1937 The distribution of gene frequencies in populations. Proc. Nat. Acad. Sci. **23**: 307–320.

WRIGHT, S., TH. DOBZHANSKY, and W. HOVANITZ, 1942 Genetics of natural populations. VII. The allelism of lethals in the third chromosome of Drosophila pseudoobscura. Genetics **27**: 363–394.

THE SIGNIFICANCE OF MULTIVALENT FORMATION IN THREE-SPECIES GOSSYPIUM HYBRIDS

MARGARET Y. MENZEL AND META S. BROWN[1]

Received December 22, 1953

FOR some years the cotton improvement program at the Texas Agricultural Experiment Station has included studies of three-species hybrids combining tetraploid New World cultivated cottons (with the genome constitution 2(AD)), Asiatic cultivated cottons (diploids of genome constitution 2A) and American wild diploid Gossypiums (2D). A fuller explanation of genome symbols, adapted from BEASLEY, is given by BROWN and MENZEL (1952b).

Earlier several investigators, including the authors, had studied the pairing relations between chromosomes of the 2A and 2D genome groups and 2(AD), but attention had been focused mainly on the completeness or incompleteness of pairing of the diploid genomes with one or the other subgenome of the tetraploid species. This work had established that at metaphase I, chromosomes of 2A species are usually completely paired with 13 chromosomes of the tetraploids, and that the other 13 chromosomes pair completely with those of the 2D species.

In the course of a study of chromosome pairing in three-species hybrids of constitution (AD)AD, in which pairing was therefore expected to approximate 26 II, it was found that modally the chromosomes did indeed show complete pairing, but that there was a high frequency of multivalent formation (BROWN and MENZEL 1950). This led to a re-examination of the literature and reanalysis of hybrids at hand of the constitution (AD)A and (AD)D. The viable (AD)D hybrids between tetraploid species and American wild diploid species all form approximately 13 II and 13 I, with an occasional III presumably composed of a D pair and an A chromosome (for summary see BROWN and MENZEL 1952a). But the $(AD)_1A_1$ *hirsutum-herbaceum* hybrid was found to have modal pairing of 9 II 2 IV 13 I while incomplete analysis of $(AD)_1A_2$, *hirsutum-arboreum*, suggested a mode of 1 IV + 1 VI per cell in addition to II's, I's and III's. Meanwhile GERSTEL (1953) reexamined the pairing relations among *G. hirsutum*, *G. arboreum* and *G. herbaceum* and concluded that *G. arboreum* and *G. herbaceum* differ by one reciprocal translocation (form 1 IV in the F_1 hybrid), *G. herbaceum* and *G. hirsutum* by two translocations (2 IV), and *G. arboreum* and *G. hirsutum* by three translocations (1 IV + 1 VI). The chromosome end arrangement of *G. herbaceum* was considered the most primitive because it was shown to be identical with that of the primitive wild species *G. anomalum*. GERSTEL has reviewed the

[1] Contribution from Department of Agronomy, Cotton Investigations Section, Texas Agricultural Experiment Station, College Station, Texas, as Technical Article No. 1871. A part of the work was done under Regional Research Project S-1.

earlier literature on (AD)A pairing. Some investigators had noted multivalent formation, but at that time evidence was being marshalled to show that the New World tetraploids are of amphiploid rather than autoploid origin, and the full significance of the multivalents was not emphasized. It was not then certain whether they were due to residual autoploidy within the A genome or to segmental interchange. Although BEASLEY (1942) referred to them as translocations, he failed to distinguish between the two IV's of A_1A_h and the IV + VI of A_2A_h.

THEORETICAL CONSIDERATIONS

GERSTEL's study makes it seem certain that the multivalents are due to segmental interchange rather than to polyploidy or duplication within the A genome. This interpretation is supported by the fact that multivalents are extremely rare in normal *G. hirsutum*. No convincing cases have been seen in this laboratory among several thousand pollen mother cells examined each year. Moreover, when the multivalents are introduced into fertile tetraploid plants, segregation for the presence and number of multivalents occurs in their progenies, as is expected in progenies of translocation heterozygotes. If the line had *G. herbaceum* as the A-genome ancestor, plants are recovered with 26 II, 24 II 1 IV, and 22 II 2 IV. If *G. arboreum* was the A-genome ancestor, plants with 26 II, 24 II 1 IV, 23 II 1 VI, and 21 II 1 IV 1 VI are obtained. That is, the two different multivalent figures segregate independently as would be expected if they were due to translocations.

The end arrangements of the five A chromosomes concerned in the translocations, as they are understood at present from all the available evidence, are represented schematically in figure 1. Assuming an initial end arrangement identical with A_1, the cytological configurations cited for A_1A_2, $(AD)A_1$ and $(AD)A_2$ can be explained simply by assuming that one reciprocal translocation occurred and became established in the evolution of A_2, and two reciprocal translocations in A_h. Divergence from the primitive end arrangement of A_1 must have occurred independently in A_2 and A_h, and to obtain the hexavalent in $(AD)A_2$ it is necessary only that one chromosome involved in A_h be one of the chromosomes involved in the A_2 translocation. The remaining eight chromosomes of all the A genomes presumably have identical end arrangements and are not shown.

We may now consider what the cytological effect of the different end arrangements of the various A genomes will be when they are combined in fertile tetraploid hybrids of the general genome constitution 2AD. Any such hybrid which has a complete set of A_h and a complete set of A_1 or A_2 chromosomes should show the same chromosome configurations as the corresponding triploid hybrid, except that the D genome I's of the triploid will now be represented by II's. Hence an $(AD)_1A_1D$ hybrid should characteristically form 22 II 2 IV at metaphase I, and an $(AD)_1A_2D$ hybrid, 21 II 1 IV 1 VI.

In practice, 2AD plants of hybrid origin have been obtained in three ways: (1) by repeatedly backcrossing a colchicine doubled (hexaploid) hybrid,

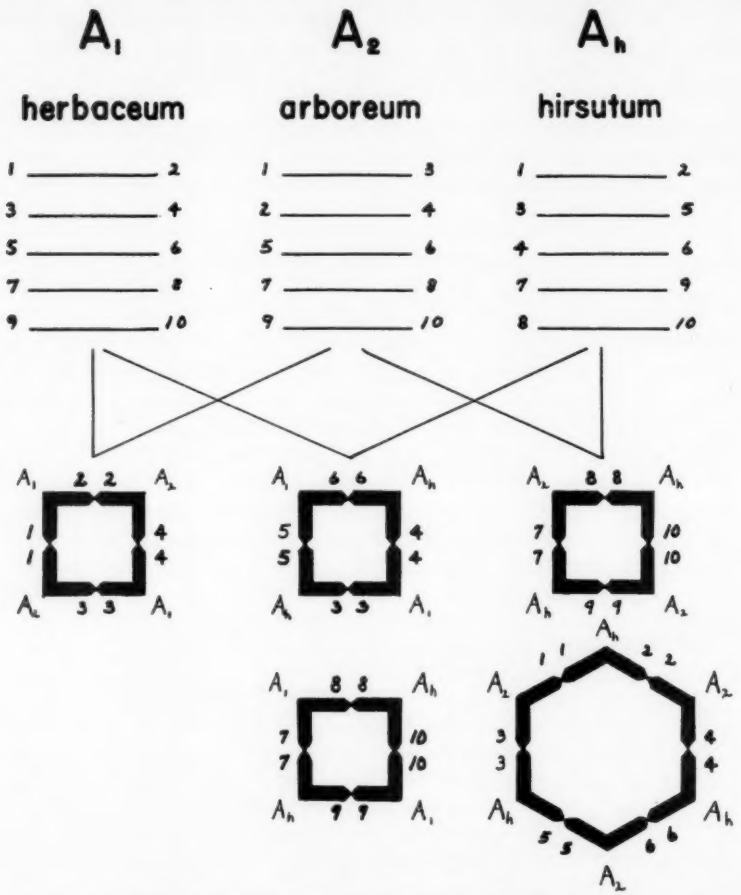

FIGURE 1.—Diagram showing the end arrangements of the differential chromosomes in *G. herbaceum*, *G. arboreum* and the A_h subgenome of *G. hirsutum*.

$2(AD)_1A_1 \times 2(AD)_1$ until the tetraploid chromosome number is restored (usually by about the third or fourth generation); (2) by crossing a synthesized 2AD allotetraploid $\times$ 2(AD); and (3) by crossing a $2(AD)_1A$ hexaploid $\times$ a 2D species. The first method combines only two species; the second and third method, three species.

When the 2AD hybrid is obtained by crossing synthesized $\times$ natural allotetraploid (method 2 above), it can be assumed, disregarding rare gametes resulting from crossovers between A and D chromosomes, that all of the F_1 hybrids have similar A-genome constitutions of 13 A_h and 13 A_1 or A_2 chromosomes. Hence all should show the full complement of multivalents due

to heterozygosity for the different A-genome end arrangements, and a maximum of 2 IV's per cell in $(AD)_1A_1D$ and of 1 IV + 1 VI per cell in $(AD)_1A_2D$ can thus be attributed to the A-genome translocations.

The situation becomes more complicated when the tetraploid hybrid is obtained by crossing a 2(AD)A hexaploid either to 2(AD) or to 2D species (methods 1 and 3). In this case, both the A_h and A_1 or A_2 components are introduced via the same gamete, i.e., are derived from the same hexaploid meiotic division. The high frequency of multivalents in these hexaploids (for review see BROWN and MENZEL 1952b) indicates that preferential pairing is not very strong in them. Therefore, although their gametes will carry approximately 13 D and 26 A chromosomes, the latter will not be composed of 13 A_h and 13 A_1 or A_2 chromosomes, but of various recombinations of these. Even if only gametes which are balanced for the various ends are functional, three conditions are possible for each of the two groups of differential chromosomes: homozygous for A_h end arrangements, homozygous for A_1 or A_2 end arrangements, or heterozygous. Since the two groups segregate independently of each other, nine different kinds of gametes are possible from either $2(AD)_1A_1$ or $2(AD)_1A_2$ if all of the differential chromosomes are considered. If duplications and deficiencies are viable, this number will of course be increased.

Therefore, the F_1 hybrids having a hexaploid as one parent will differ from each other in their A_h and A_1 or A_2 composition unless there is very strong selection for a single kind of functional gamete in the hexaploid. This point may be tested by comparing the multivalent configurations in individual F_1 plants and in their first backcrosses to *G. hirsutum*.

Data have accumulated over a period of years on metaphase pairing in hybrid material obtained by all three of the methods considered above. They will be examined here with a view to answering the following questions: (a) Can the A_1 and A_2 end arrangements be transferred to an essentially *hirsutum* background? (b) Can all of the multivalent formation in $(AD)_1AD$ three-species hybrids be attributed to the A-genome translocations? (c) Do three-species hybrids obtained via hexaploid × diploid crosses differ from each other and from hybrids from tetraploid × tetraploid crosses with regard to the A-genome chromosomes involved in the translocations? (d) What is the behavior of the multivalents in subsequent generations, and what bearing may they be expected to have upon the breeding behavior of species-hybrid derivatives?

MULTIVALENTS FROM HIRSUTUM-ASIATIC PENTAPLOIDS

Data from backcrosses of the *hirsutum*-Asiatic hexaploids to *hirsutum* were collected incidental to recovery and study of trisomic lines and without particular effort to isolate or maintain the translocations. Reexamination of these data allows the following conclusions, however: (1) All of the multivalent formation in the pentaploid and later generations (above that attributable to aneuploidy) can be accounted for by the A-genome translocations. No pairing

between A and D genomes need be postulated. (2) Lines carrying single translocation multivalents which subsequently behave cytologically as if they were reciprocal translocations within *G. hirsutum,* are readily isolated in plants having only 52 chromosomes. As cytogenetic tools, these lines have the advantage over induced translocations in *G. hirsutum* that they are already known to involve the A_h rather than the D_h subgenome. Several such lines from both *arboreum* and *herbaceum* have been established and are being tested against each other and against cytologically aberrant *G. hirsutum* lines to establish whether the same or different chromosomes are involved.

MULTIVALENTS IN TETRAPLOID × TETRAPLOID THREE-SPECIES HYBRIDS

Metaphase I pairing in the *arboreum-thurberi-hirsutum* hybrid, A_2D_1-$(AD)_1$, was previously reported in some detail (BROWN and MENZEL 1950, table 1). Reference to this report shows that only two out of 58 PMC's analyzed showed the expected pairing of 21 II 1 IV 1 VI. Two modes lay at 23 II 1 VI and 24 II 1 IV (5 and 6 cells respectively). Since it is not expected that all of the possible chiasmata will form in every cell in a translocation heterozygote, multivalent formation of 1 IV + 1 VI *or less* can be considered to be due to the A-genome translocations. Only 33 out of the 58 cells analyzed can be so accounted for. The remaining cells show evidence of excess multivalent formation. There is no evidence that any of the D genomes differs structurally from the D_h subgenome in such a way as to give multivalents. But there is considerable evidence that varying amounts of residual homology exist between various A and various D genomes. Metaphase pairing ranges from an average of 1.5–1.7 associations of two chromosomes per cell in *G. hirsutum* haploids (BEASLEY 1942; BROWN and MENZEL 1952a) to about 4.7 (BEASLEY 1942) to 8 (SKOVSTED 1937) in A_2D_1 F_1 hybrids. Hence it may be concluded that excess multivalent formation is due to chiasmata between A and D chromosomes. This conclusion is supported by the appearance of some of the multivalents at metaphase I. The A chromosomes are on the whole somewhat larger than the D chromosomes. Although this size difference is neither large enough nor constant enough to be used as a precise criterion in all preparations, it can often be used as an indication of the genome affinity of a chromosome or group of chromosomes. Notes taken during study of the $A_2D_1(AD)_1$ hybrid, before the present hypothesis regarding multivalent formation was formulated, indicated that many of the multivalents were composed entirely of large chromosomes, but that some were composed partly of large and partly of small chromosomes.

In the $A_2D_1(AD)_1$ hybrids, not more than three chiasmata between A and D chromosomes need be postulated to account for pairing in any of the cells recorded; 18 of the 25 cells can be accounted for by assuming only one AD chiasma. It is of interest that this frequently seems to involve pairing between a D II and the A_hA_2 VI. In 10 of the 25 cells, either an VIII, a VII + I, or 1 III + 1 V without I's was formed. Some of the other configurations may also be explained in this way if the potential VIII of 6 A and 2 D chromosomes

forms, instead, 2 IV's, 1 III + 1 IV + 1 I, etc. The presence of an association of 10 chromosomes in one cell suggests that the D II also has some homology with one of the A_hA_2 II's, perhaps in the opposite arm. The frequent involvement of the VI in AD pairing suggests that the intergenomic pairing is not at random, but rather confined to only a few segments of the D genomes.

MULTIVALENTS IN HEXAPLOID × DIPLOID THREE-SPECIES HYBRIDS

In the previous report of pairing in *hirsutum-arboreum-harknessii,* $(AD)_1A_2D_{2\text{-}2}$, and *hirsutum-herbaceum-harknessii,* $(AD)_1A_1D_{2\text{-}2}$ (BROWN and MENZEL 1950), data from several plants were combined. Sufficient cells from five different hexaploid × diploid F_1 plants have been analyzed to allow them to be considered separately (table 1). In all five of these plants, the average number of chromosomes per cell participating in multivalent formation was lower than in the hybrids obtained by tetraploid × tetraploid crosses. In all five, however, some cells showed multivalents in excess of those ascribed to A-genome translocations, ranging from only one cell (2.5%) in one $(AD)_1A_2D_{2\text{-}2}$ hybrid to 36% of the cells in the $(AD)_1A_1D_{2\text{-}2}$ hybrid. In all five, the estimated frequency of AD pairing was lower than in the $A_2D_1(AD)_1$ hybrids.

The lower multivalent frequency in the five hexaploid × diploid hybrids suggests that none of them was heterozygous for all of the chromosomes involved in the translocations.

MULTIVALENTS IN F_2 AND BACKCROSSES FROM THREE-SPECIES HYBRIDS

A summary of pairing in first backcross progenies of two different hexaploid × diploid $(AD)_1A_2D_{2\text{-}2}$ hybrids is shown in table 2. Plants from F_1 Z593 (which was not analyzed) probably all had the IV (one plant was doubtful), while four did and three did not have the VI. Plants from Z595 × *hirsutum* all exceeded Z595 in multivalent formation, and of 21 plants, all but one or two had the IV, and all had the VI, despite the fact that only two VI's were seen in 25 PMC's of the parent. These findings indicate that Z593 and Z595 did not have identical A_2A_h constitution, and that Z595 at least was probably a duplication-deficiency type which had become homozygous for one or more of the A_2 end arrangements. (Had it been homozygous for A_h end arrangements, multivalent formation should not have increased on backcrossing to *G. hirsutum*). Also of interest is the fact that some of the first backcross plants exceeded their parent in frequency of AD chiasmata, and approached or equaled the $A_2D_1(AD)_1$ hybrids in frequency and complexity of multivalent formation. It will be noted that apparently none of the 28 first backcross plants had returned completely to the *G. hirsutum* end arrangement. All showed at least one translocation configuration.

Unfortunately, insufficient first backcross plants from tetraploid × tetraploid $A_2D_1(AD)_1$ have been analyzed to permit a direct comparison. Table 3, however, summarizes the pairing in 16 F_2 plants from this hybrid. Seven to ten plants had both IV and VI, two had only the VI, three only the IV, and one trisomic plant had neither. With the exception of one plant from which

TABLE 1

Comparison of metaphase pairing in three-species Gossypium hybrids of genome constitution (AD)AD.

Plant No.	Type of cross	Genome symbol	No. PMC	Av. No. chromosomes per cell paired as multivalents[b]	Cells with excess multivalents		AD chiasmata per cell	
					No.	%	Mode	Max.
Combined data, several plants	Tetraploid × tetraploid	$A_2D_1(AD)_1$	58	9.80	25	43.1	1	3
Z905	Hexaploid × diploid	$(AD)_1A_2D_1$[c]	11	7.45	3	27.2	1	2
Z594	Hexaploid × diploid	$(AD)_1A_2D_{2-2}$	42	5.74	1	2.5	1	1
Z595	Hexaploid × diploid	"	25	4.64	4	16.0	1	1
Z596	Hexaploid × diploid	$(AD)_1A_1D_{2-2}$	25	5.76	9	36.0	1	4
Z907	Hexaploid × diploid	$(AD)_1A_2D_5$[a]	28	7.89	4	14.3	2	2

[a] D_5 = *G. raimondii* genome.
[b] Including trivalents.
[c] Hybrid made by J. R. Meyer.

TABLE 2

TABLE 2

Pairing in the first backcross generation from two different $(AD)_1A_2D_{2-2}$ F_1 hybrids × G. hirsutum.

Parent	Plant No.	Direction of cross[a]	No. PMC analyzed	A-genome translocation multivalents present		AD pairing		
				IV	VI	No. cells	%	Max. AD Xmata
A. Z593	52C-1-1950	♂	38	+	+	2	5.2	2
	52H-3-1950	♀	35	+	+	10	28.5	2
	426B-2-1951	♂	17	+	+	3	17.6	2
	Z869	♀	22	?	+	?	?	?
	Z868	♂	17	+	....	....	....	....
	52C-3-1950	♂	10	+	....	4	40.0	1
	52C-4-1950	♂	20	+	....	....	....	....
B. Z595	Z755	♀	26	+	+	1	3.8	1
	Z756	♀	33	+	+	....	....	....
	Z759	♀	29	+	+	2	6.9	1
	Z760	♀	28	+	+	6	21.4	1
	Z761	♀	25	+	+	3	12.0	1
	52E-1-1950	♂	34	+	+	3	8.8	1
	52F-1-1950(2n + 1)	♀	33	+	+	?[b]	?[b]	?[b]
	52F-2-1950	♀	25	+	+	8	35.0	1
	52G-2-1950	♀	30	+	+	3	10.0	1
	52G-3-1950	♀	25	+	+	2	8.0	1
	52I-1-1950	♀	18	+	+	6	33.3	1
	52I-2-1950	♀	26	+	+	8	30.8	2?
	52I-3-1950	♀	17	+	+	2	11.7	1
	53A-1-1950	♀	20	+	+	4	20.0	1
	53A-2-1950	♀	28	+	+	5	17.7	3
	Z891	♂	33	+	+	11	33.3	2
	426A-2-1951	♂	8	+	+	2	25.0	1
	426A-4-1951	♂	31	+	+	....	....	....
	426C-1-1951	♀	9	+	+	....	....	....
	Z757	♀	22	?	+	....	....	....
	52G-1-1950[c]	♀	23	....?	+?	1	4.3	1

[a] Symbol indicates whether the F_1 hybrid was ovule or pollen parent.

[b] This 53-chromosome plant gave complex pairing, one cell showing maximum pairing of 1 III, 1 IV, 1 chain of X.

[c] This plant probably lacked the IV and had a duplication-deficiency from the VI. It showed modal pairing (11 cells) of 1 IV + 2 I, but some cells had a VI.

TABLE 3

Pairing in F_2 plants from arboreum-thurberi-hirsutum.

Plant No.	No. PMC	IV	VI	AD pairing		
				No. cells	%	Max. AD Xmata
139B-2-1949	16	+	+	2	12.5	1
140A-6-1949(2n + 1)	13	+	+	3	23.0	4
141A-6-1949	15	+	+	2	13.3	1
141A-7-1949(2n + 1)	8	+	+	1	12.5	1
194A-3-1949	26	+	+	10	38.4	4
292A-19-1949(2n + 1)	19	+	+?	....	....	....
Z696	20	+	+	5	25.0	1
Z697	25	+	+	8	32.0	2
140A-2-1949	9	+?	+	?	?	?
194A-6-1949(2n + 1)	24	+?	+	....	....	....
141A-3-1949	12	....	+	4	33.3	1
292A-13-1949	5	....	+	5	100.0	1
141A-13-1949	56	+	....	6	10.7	1
194A-4B-1949	6	+	....	....	....	....
291B-13-1949	36	+	....	5	13.9	1
292A-15-1949(2n + 1)	28	....	....	....	....	....

only five cells were analyzed (four having 22 II 1 VIII and one, 22 II 2 IV), all the F_2 plants had a lower frequency of cells with AD chiasmata than the F_1. Since the backcross plants listed in table 2 indicate that both the A_h and A_2 end arrangements may pass through both pollen and ovules, it should be pointed out that in the F_2, when one of the translocation configurations had been lost, the plant may have become homozygous for either the A_h or the A_2 end arrangement.

DISCUSSION

Experience with *hirsutum-barbadense* and with three-species hybrids in Gossypium, recently reviewed by RICHMOND (1951), has shown that when species hybrids are incorporated into a breeding program, certain difficulties are encountered which are not present in intraspecific breeding. These difficulties do not indicate that such programs cannot succeed, but rather that much more information is needed concerning the nature of the barriers which interfere with free recombination of desirable traits and the ready transference of species-foreign characters to an otherwise *hirsutum* background. At least two hypotheses have previously been proposed to explain these barriers, the " multiple gene substitution " theory of HARLAND (1936) and " cryptic structural hybridity " in the sense of STEBBINS (STEPHENS 1950). With regard to the three-species hybrids, it is now necessary to consider also the possible effects of gross differences in chromosome structure.

We are now in a position to give tentative answers to the questions which were raised above:

(a) The differential chromosome end arrangements of A_1 and A_2 can be maintained indefinitely, at least in heterozygous condition, on an essentially *hirsutum* background.

(b) Data at hand show that the A-genome translocations can account for all the multivalent formation in backcrosses from the *hirsutum*-Asiatic hexaploids (above that due to aneuploidy), and for a major portion, but not all, of that in the three-species hybrids. The excess multivalents in the latter must be attributed to pairing between A and D chromosomes. It was estimated that as high as 43% of PMC's may show from one to four such intergenomic chiasmata. It should be pointed out that this estimate is a minimum value. It is probable that the actual frequency is somewhat higher. The frequent occurrence of AD chiasmata was not expected from what was known concerning (AD)A and (AD)D triploid hybrids, *hirsutum*-Asiatic pentaploids, and preferential pairing in general in the genus.

(c) The available data strongly indicate that F_1 three-species hybrids derived from hexaploid × diploid crosses are not uniform in A-genome composition, and may not be assumed to be exactly equivalent to similar hybrids obtained by crossing synthesized × natural allotetraploids. This in turn suggests that if breeding is to proceed from such hybrids, selection of parents can and should begin in the F_1 generation.

(d) Probably the translocations can persist, unless intentionally selected against, for some time in breeding material, especially if selection for high fertility has not been the main objective. For instance, when a second backcross plant from the *arboreum-thurberi-hirsutum* hybrid, selected for high fiber strength, was selfed, only one out of seven plants analyzed cytologically had 26 II. One still retained the full A_hA_2 multivalent complex of 1 IV + VI, while the others showed at least one multivalent configuration. Thus if a breeding line has been selfed, one is not certain that it is homozygous for the *hirsutum* end arrangements, even though it shows only 26 II at metaphase. And even if it has been backcrossed two or more times, it may not be assumed without cytological confirmation that all the *hirsutum* end arrangements have become homozygous.

Moreover, some of the data suggest that some of the duplication-deficiency gametes from the A-genome translocations may be functional (as they are in at least one induced reciprocal translocation in *G. hirsutum*, MENZEL and BROWN 1952). The existence and significance of these types remain to be verified; if present, they may have considerable bearing on some of the complications that arise in the breeding work.

It is not yet possible to estimate how much the A-genome translocations interfere with fertility in the derivatives of three-species hybrids, but it is likely that they reduce it to some extent, both through non-disjunction and by retaining large unbroken blocks of species-foreign genic material.

In many instances in which the (AD)AD three-species hybrids have been used in breeding programs, the primary intention has been to introduce into *G. hirsutum* characters from the American wild ancestor. The A-genome translocations should not interfere with the transference of chromosome segments from the D ancestor. However, the fairly high frequency of excess multivalents, deduced to be due to AD chiasmata, may do so. Assuming that they represent crossovers, such chiasmata can give rise in backcrosses to

G. hirsutum to a variety of cytological aberrations, including new reciprocal translocations between A and D chromosomes, and possibly also to insertions of D segments into A chromosomes or vice versa, where, with continued backcrossing to *hirsutum* (in which AD pairing does not occur) they may become "trapped" and difficult to alter or eliminate by crossing over.

It appears that the irregularities of meiosis in the F_1 three-species hybrids may be primarily of nuisance value for practical breeding purposes. In this regard, it would be of importance to know how quickly they may be eliminated without also eliminating the species-foreign characters which it is desired to retain. These aberrations are otherwise of interest, however, as a source of cytological types for use in basic study of the comparative cytogenetics of Gossypium species, since they may be expected to include several different kinds of translocations, deficiency-duplications, substitution races, and possibly other types not yet recognized.

SUMMARY

Demonstration that the A_h subgenome of *G. hirsutum* differs from the A_1 (*G. herbaceum*) genome by two, and from the A_2 (*G. arboreum*) genome by three reciprocal translocations led to re-evaluation of multivalent formation in three-species Gossypium hybrids combining *G. hirsutum, G. herbaceum* or *G. arboreum,* and American wild diploid species (D genome). The A-genome translocations account for all the multivalent formation not due to aneuploidy in *hirsutum*-Asiatic pentaploids and their descendants. Excess multivalent formation occurring in the three-species hybrids and their progeny is attributed to chiasmata between A and D chromosomes. As high as 43% of pollen mother cells may show from one to four AD chiasmata at metaphase I. Three-species F_1 hybrids obtained by crossing 2(AD)A hexaploids × 2D species differ from each other both in their complement of A-genome end arrangements and in the frequency of AD chiasma formation. Some first backcross plants from such hybrids exceed their parents in multivalent formation of both types. Implications of A-genome multivalent formation and of AD chiasmata are discussed. Although the A-genome translocations may be recovered in heterozygous condition even after several backcrosses to *G. hirsutum,* they may also be rather quickly eliminated if deliberately selected against. The possible consequences of the rather high frequency of AD pairing remain to be verified. In addition to the hypotheses of "multiple gene substitution" and "cryptic structural hybridity," previously advanced to account for problems peculiar to interspecific breeding in Gossypium, use of the three-species hybrids must also take into account the gross structural differences already existing between the A genomes, and the possibility of newly-arising rearrangements resulting from chiasmata between A and D chromosomes.

ACKNOWLEDGMENTS

The authors wish to thank DR. T. R. RICHMOND for his interest and encouragement in this study.

LITERATURE CITED

BEASLEY, J. O., 1942 Meiotic chromosome behavior in species, species hybrids, haploids and induced polyploids of Gossypium. Genetics **27**: 25–54.

BROWN, META S., and MARGARET Y. MENZEL, 1950 New trispecies hybrids in cotton. J. Hered. **41**: 291–295.

1952a The cytology and crossing behavior of *Gossypium gossypioides*. Bull. Torrey Bot. Club **79**: 110–125.

1952b Polygenomic hybrids in Gossypium. I. Cytology of hexaploids, pentaploids and hexaploid combinations. Genetics **37**: 242–263.

GERSTEL, D. U., 1953 Chromosomal translocations in interspecific hybrids of the genus Gossypium. Evolution **7**: 234–244.

HARLAND, S. C., 1936 The genetical conception of the species. Biol. Rev. **11**: 83–112.

MENZEL, MARGARET Y., and META S. BROWN, 1952 Viable deficiency-duplications from a translocation in *Gossypium hirsutum*. Genetics **37**: 678–692.

RICHMOND, T. R., 1951 Procedures and methods of cotton breeding with special reference to American cultivated species. Adv. in Genetics **4**: 213–245.

SKOVSTED, A., 1937 Cytological studies in cotton. IV. Chromosome conjugation in interspecific hybrids. J. Genet. **34**: 97–134.

STEPHENS, S. G., 1950 The internal mechanism of speciation in Gossypium. Bot. Rev. **16**: 115–149.

X-RAY-INDUCED DOMINANT LETHALS IN DROSOPHILA ROBUSTA [1,2]

ARMON F. YANDERS [3]

Department of Zoology, University of Nebraska, Lincoln, Nebraska

Received December 22, 1953

IT has been noted previously (YANDERS 1952) that slightly higher than expected values for induced dominant lethals and lower values for viable chromosome aberrations were obtained from the exposure of mature sperm of *Drosophila robusta* to X-rays. Three experiments involving exposure of adult males to 5,000, 7,500 or 10,000 r of X-rays yielded the results given in table 1, in which is listed the fraction of progeny surviving to adulthood, expressed as a percentage of survival in concurrent nonirradiated controls. Approximate survival values for *D. melanogaster* at these doses (from the curve of CATCHESIDE and LEA 1945) are given for comparison.

TABLE 1

Percent of progeny surviving to adulthood following the exposure of D. robusta males to X-rays; results of three experiments.

Experiment	5,000 r	7,500 r	10,000 r
1	16.16	2.65	0.16
2	10.39	2.93	0.55
3	11.32	2.43	0.21
Mean	12.13	2.68	0.33
*D. melanogaster**	25.00	9.00	2.00

*Approximate values; from the curve of CATCHESIDE and LEA (1945).

These data indicate a more pronounced reduction of fertility at each dose than that reported for other species. However, salivary gland analysis of one hundred twenty-six larvae from the 5,000 r groups yielded only eight detectable aberrations, a frequency of 6.3%. This seems extremely low when compared to figures obtained in other species at comparable doses, e.g., 31.9% (HELFER 1941, with *D. pseudoobscura*) to 44.25% (BAUER 1939, with *D. melanogaster*). If the correlation between viable aberrations observed and total aberrations induced is similar in *D. robusta* and other species, it would seem that fewer aberrations are induced in *D. robusta*. However, this is not likely to be the case, for dominant lethals themselves are most probably chromosome aberrations (MULLER 1940) and occurred at a frequency greater than expected.

[1] Prepared from portions of a dissertation presented to the Faculty of the Graduate College of the University of Nebraska in partial fulfillment of the requirements for the degree of Doctor of Philosophy.

[2] Contribution No. 269 of the Department of Zoology and Anatomy, University of Nebraska.

[3] Present address: Department of Biological Sciences, Northwestern University, Evanston, Illinois.

Since noticeable increases in the frequency of dominant lethals induced by X-rays correlated with an increase in the age of the sperm at the time of irradiation have been reported in *D. melanogaster* (e.g., DEMPSTER 1941; STRÖMNAES 1949), it seemed desirable to investigate a possible effect of age. The *D. robusta* males used had been aged eight to ten days at the time of irradiation. This particular age at first was chosen because the longer developmental period of this species, as compared to *D. melanogaster,* indicated that a longer period after eclosion must be necessary to attain full fertility. Subsequent tests demonstrated that *D. robusta* males reach fertility no sooner than eight days after eclosion, with the onset of fertility (as demonstrated by successful insemination of mature females) being delayed in some individuals until ten days or more after eclosion. Since an effect of age on X-ray sensitivity had not previously been reported with this species, it remained to be demonstrated whether even greater aging of *D. robusta* males would further increase the percentage of dominant lethals.

MATERIALS AND METHODS

A stock of *Drosophila robusta* STURTEVANT 1916, of Standard gene arrangement, kindly supplied by DR. H. L. CARSON, of Washington University, was used exclusively. The flies of this species are among the largest in the genus Drosophila, and have a relatively long life cycle, requiring more than twenty-four days at 23°C—more than twice as long as *D. melanogaster.* Two groups of males, aged ten days or seventeen days, were exposed to 0 (control), 2,500 or 5,000 r of X-rays. The source was a Westinghouse Quadrocondex Type C deep X-ray therapy machine operating at 200 kv, 18 ma, with 0.25 mm Cu + 1 mm Al filtration. The output, calibrated immediately before exposure with a Model D Victoreen dosimeter and corrected for temperature and atmospheric pressure, was 840 r per minute at 15 cm target distance.

Immediately after exposure, the males from each exposure group were mass-mated to females aged seventeen days. Eggs were collected at twenty-four-hour intervals for ten days after exposure and placed in vials in groups of twenty-five, one hundred eggs being collected from each of the six groups per day. Each vial was inspected for its initial five days to determine egg hatch, although in no case was a hatching recorded beyond the fourth day. One thousand eggs were introduced per group.

Adult flies were removed and counted soon after eclosion. Determinations of puparia number were made one week after the last adult had been recovered from each vial.

RESULTS AND DISCUSSION

The survival at each developmental stage, expressed as the percent of eggs introduced, is given in table 2. These percentages, corrected for the mean rate of egg hatch of the control of each age group, are represented graphically in figure 1. It is apparent that the lower percentage of survival previously found in *D. robusta* was most probably due to errors involving the use of adult

TABLE 2

Percent of progeny of irradiated and non-irradiated D. robusta males of two different ages attaining larval, pupal or adult stages.

Treatment	Age of males	Larvae	Pupae	Adults
Control	10 days	74.6	38.7	38.2
	17 days	85.4	41.9	39.9
2,500 r	10 days	44.8	21.6	20.6
	17 days	24.6	14.6	12.8
5,000 r	10 days	16.4	7.8	7.6
	17 days	3.6	1.9	1.8

emergence as a measure of the percent of survival. The survival of eggs fertilized by the 10-day-old treated males differs but little from that reported for *D. melanogaster* at similar dosages; but the number of progeny surviving to adulthood consists of only about fifty percent of the egg hatch in controls and treated groups alike. This is due almost entirely to death in the larval stages, with only a small mortality occurring after pupation. Since the amount of food present in the vials was capable of supporting the development of twenty-five flies, and the mortality occurred in similar ratios in vials containing few or many individuals, insufficient food is not responsible. HERSKOWITZ (1953) has noted only 52% survival from larva to eclosion in *D. simulans,* and suggests that the mortality is due to submergence of the larvae in the food, or displacement of pupae, so that successful eclosion is difficult or impossible. It is not known if this situation exists with *D. robusta,* but the mortality is independent of the irradiation.

X-ray-induced dominant lethals in *D. robusta,* as in other species, apparently act very early in embryological development. This is most strikingly evident in figure 1. If an appreciable proportion of the dominant lethals induced were to act during either the larval or pupal stages, the points indicating the mean survival at successive stages would tend to diverge as the dosage increased. The fact that they do not diverge indicates that substantially all of the lethals induced have been expressed by the failure of the eggs to hatch. Larval and pupal mortality must be due to other causes.

Analyses of variance of the data from each developmental stage indicate that the variance due to (a) treatment, (b) age of male parent at the time of irradiation, and (c) increase of fertility with time past treatment, is highly significant in each case (table of distribution of F as given by SNEDECOR 1946); but because of the high degree of mortality in the larval stage, it is best to confine the discussion to the percent of egg survival. The analysis is as follows:

Source	D.F.	S.S.	M.S.
Total	239	16293.184	
Treatment (a)	2	12603.634	6301.817**
Age (b)	1	205.350	205.350**
Days (c)	9	1386.767	154.085**
Error	227	2098.433	9.244

The results show conclusively that the age of the male fly at treatment is an important factor affecting the percentage of dominant lethals induced. Although the percentage of fertility was higher in the 17-day controls than in the 10-day controls, irradiation more greatly reduced the fertility of the 17-day groups in both absolute percentage and percentage relative to controls.

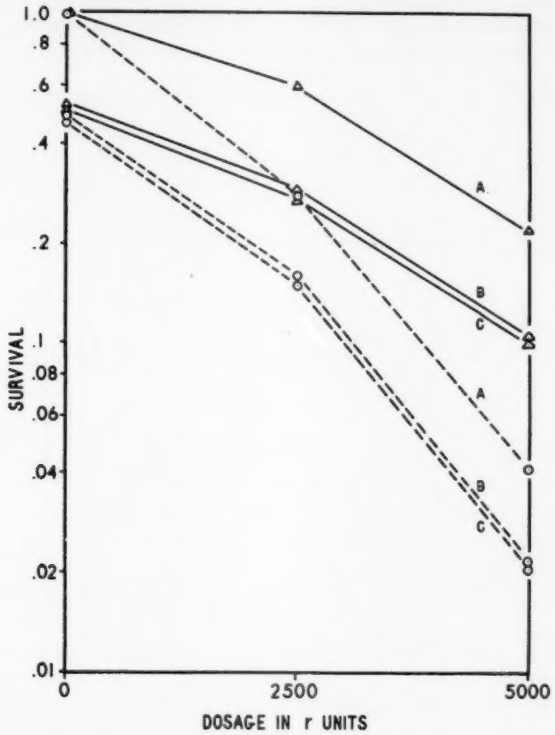

FIGURE 1.—The occurrence of dominant lethals in X-rayed sperm of *D. robusta* males of two different ages. Semi-logarithmic representation: Abscissa = dosage in r; Ordinate = fraction of survival to (A) hatching, (B) pupation and (C) eclosion. Solid lines = progeny of males ten days old at irradiation; Broken lines = progeny of males seventeen days old at irradiation.

It appears from these data that relatively younger postmeiotic germ cells are less sensitive than those which are older, since the percentage of egg fertility rose gradually until the conclusion of the experiment at ten days past treatment. Regression lines for each age-dose, corrected for the regression in controls, are given in figure 2. That this increase in fertility is real is shown also by the analysis of variance (tested under " Days " above). It is possible to interpret the rise in fertility as reflecting an increased use of sperm which

were relatively immature at treatment, the sperm mature at treatment having been replaced by progressively younger sperm; but BAKER and VON HALLE (1953), who also have reported such a phenomenon in *D. melanogaster*, discount differential sensitivity. They demonstrated that the older sperm, when retained by the male for a period of twenty-four hours, themselves exhibit a decreased value for dominant lethals; this lower value is equivalent to that obtained for sperm which were twenty-four hours younger at irradiation. They attribute the decrease to restitution of induced breaks taking place in the male

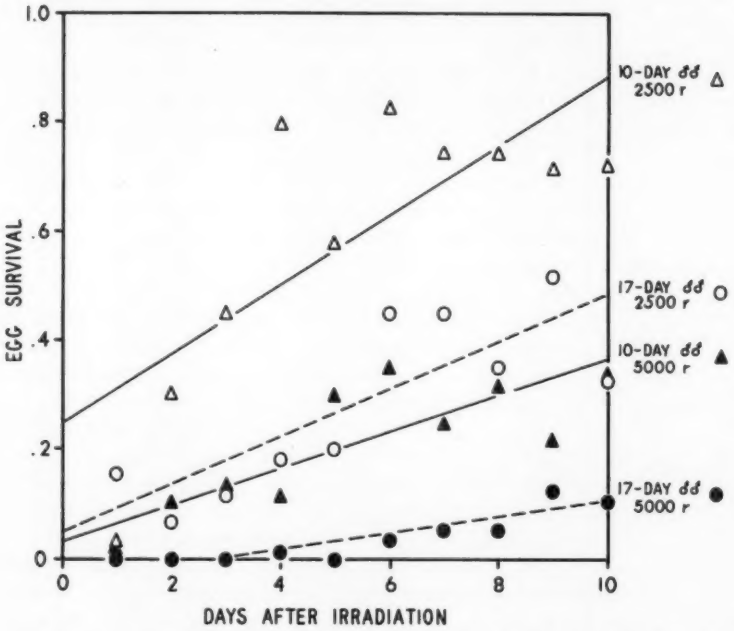

FIGURE 2.—Linear regression of survival of eggs fertilized by irradiated sperm plotted against the day of collection, irradiation occurring on day 0. Abscissa = day of collection of eggs; Ordinate = fraction of egg hatch, corrected for the regression occurring in controls.

prior to insemination of the female. Their hypothesis would explain the results obtained with *D. robusta*; and, if it is assumed that either restitution takes place at a faster rate in younger flies, or that chromosome breakage is greater in older flies (or a combination of both), the regression lines of figure 2 give a consistent pattern. It must be remembered, however, that mass cultures were employed for the collection of eggs, so that the eggs laid during a twenty-four-hour period may have contained sperm of various ages, as well as unfertilized eggs laid by virgin females; but it seems that the values obtained are indicative of a trend which would be even more obvious using a more elegant technique.

The results parallel the findings of DEMPSTER (1941) and STRÖMNAES (1949) that increases in age in *D. melanogaster* lead to greater sensitivity to X-rays, with corresponding increases in the production of dominant lethals. BONNIER and LÜNING (1950) also have demonstrated that the rate of dominant lethals varied with the age of males at treatment; and LÜNING (1952) has obtained two distinct regression lines for hatchability when various doses of X-rays were given to *D. melanogaster* males aged zero to one, and six to seven days. LÜNING concluded that the results of all of these studies agree with the hypothesis of a different breakability in the chromosomes of males of different ages, and in different stages of spermiogenesis, the relative susceptibility being (from highest to lowest): immature sperm of 0–1-day-old males; immature sperm of 6–7-day-old males; mature sperm of 6–7-day-old males; mature sperm of 0–1-day-old males. In LÜNING's study, the apparent increased sensitivity of immature sperm is evident from an increase in the percentage of dominant lethals in sperm inseminated 7–10 days following irradiation.

The results obtained in the present study agree with the hypothesis of a greater breakability in the mature sperm of older males. However, in *D. robusta,* a consistent decrease in the percent of dominant lethals was noted throughout the ten-day period, with no subsequent rise corresponding to that in *D. melanogaster.* This situation most probably reflects the slower rate at which physiological processes proceed in *D. robusta,* as evidenced by their longer life cycle and greater period from eclosion to sexual maturity. If we assume that all processes, including spermiogenesis, take place at this slower speed, we see essentially in the ten days following irradiation of *D. robusta* that which occurs in *D. melanogaster* during the first day or two after irradiation. During this time, some of the chromosomes broken by irradiation restitute prior to fertilization, and all restitution which takes place effectively decreases the proportion of dominant lethals. Such a continuous restitution process also will account for the apparently smaller number of detectable aberrations in *D. robusta* (noted above), since the number of breaks available for the formation of rearrangements becomes smaller on succeeding days following irradiation.

SUMMARY

Drosophila robusta males, aged ten days or seventeen days, were exposed to 0 (control), 2,500 or 5,000 r of X-rays and mass-mated. Eggs were collected at twenty-four-hour intervals for a period of ten days, and records of egg fertility, formation of pupae, and emergence of adult progeny were obtained.

Values for induced dominant lethals in the groups aged ten days are similar to the values for *D. melanogaster* at corresponding dosages. Sperm of males aged seventeen days, however, exhibit a greater sensitivity, agreeing with the hypothesis of a greater breakability of chromosomes in sperm of aged males.

A gradual decrease in the number of dominant lethals was noted over the ten-day period following irradiation; it is suggested that restitution of induced

breaks is responsible. The fact that this increase in fertility is noted throughout the ten-day period over which eggs were collected, with no subsequent drop as noted after seven days with *D. melanogaster*, may indicate that physiological processes involved in spermiogenesis occur at a slower rate in *D. robusta* than in *D. melanogaster*.

ACKNOWLEDGMENT

The writer wishes to express his appreciation to PROFESSOR DWIGHT D. MILLER, University of Nebraska, Department of Zoology, for his counsel and encouragement; to PROFESSOR C. T. BLUNN, University of Nebraska College of Agriculture, Department of Animal Husbandry, for his suggestions concerning the statistical analysis; and to MR. A. T. TUMA of the Department of Radiology, University of Nebraska College of Medicine, Omaha, Nebraska, for his conscientious performance of the X-irradiation.

The study was made with the aid of an Atomic Energy Commission Predoctoral Fellowship in the Biological Sciences.

LITERATURE CITED

BAKER, W. K., and E. S. VON HALLE, 1953 The basis of the oxygen effect on X-irradiated Drosophila sperm. Proc. Nat. Acad. Sci. **39**: 152–161.

BAUER, H., 1939 Röntgenauslösung von Chromosomenmutationen bei *Drosophila melanogaster*. Chromosoma **1**: 343–390.

BONNIER, G., and K. G. LÜNING, 1950 X-ray-induced dominant lethals in *Drosophila melanogaster*. Hereditas **36**: 445–456.

CATCHESIDE, D. G., and D. E. LEA, 1945 The rate of induction of dominant lethals in *D. melanogaster* sperm by X rays. J. Genet. **47**: 1–9.

DEMPSTER, E. R., 1941 Dominant vs. recessive lethal mutation. Proc. Nat. Acad. Sci. **27**: 249–250.

HELFER, R. G., 1941 A comparison of X-ray-induced and naturally occurring chromosomal variations in *Drosophila pseudoobscura*. Genetics **26**: 1–22.

HERSKOWITZ, I. H., 1953 The survival and developmental rates of *D. melanogaster* and *D. simulans*. Amer. Nat. **87**: 113–114.

LÜNING, K. G., 1952 X-ray-induced chromosome breaks in *Drosophila melanogaster*. Hereditas **38**: 321–338.

MULLER, H. J., 1940 An analysis of the process of structural change in the chromosomes of *Drosophila melanogaster*. J. Genet. **40**: 1–66.

SNEDECOR, G. W., 1946 Statistical Methods. Ames, Iowa: Iowa State College Press: xvi + 485 pp.

STRÖMNAES, ÖISTEIN, 1949 The production of dominant lethals with X rays in aged *Drosophila melanogaster* sperm. Genetics **34**: 462–474.

YANDERS, A. F., 1952 X-ray-induced chromosome aberrations and dominant lethals in *Drosophila robusta*. Rec. Gen. Soc. Amer. **21**: 82.

CHROMOSOME LOSS CAUSED BY ULTRAVIOLET TREATMENT OF DROSOPHILA SPERMATOZOA[1]

JOHN T. McQUATE[2]

Zoology Department, Indiana University, Bloomington, Indiana[3]

Received December 28, 1953

THE discovery by MULLER (1927) that gene mutations could be induced in Drosophila by the administration of X-radiation was followed by ALTENBURG's finding (1930 *et seq.*) that ultraviolet was also capable of inducing mutations in the gene. In contrast, however, to the discovery that X-radiation also produced many chromosome rearrangements, the results from ultraviolet treatment indicated comparatively few changes of such kinds.

ALTENBURG in 1930 obtained a few translocations, but did not publish these results (see review by MULLER 1954). MULLER and MACKENZIE (1939), and MACKENZIE and MULLER (1940) made extensive tests for translocations and although they obtained an average lethal frequency of 3.47% in the X chromosome a test of 5,042 treated sperm failed to reveal a single translocation. Since a dose of X-rays giving such a lethal frequency in the X would have at the same time produced some 65 translocations, this difference between 0 and 65 was highly significant, and seemed to indicate that ultraviolet light was incapable of producing chromosome rearrangements. SLIZYNSKI (1942), however, in the cytological analysis of the salivary chromosomes of 21 of the ultraviolet-induced lethals obtained by MULLER and MACKENZIE, reported 1 deficiency with 1 band deficient, 3 with 2 bands deficient (all these being of sizes which some cytologists regard as too small for certainty of recognition), and 1 with 14 bands deficient. Moreover, MULLER (1941) did obtain one translocation in ultraviolet-treated material. This was detected as a sex-linked semi-lethal, and was a translocation between the X and the 2nd chromosomes. He also obtained one small deficiency in the X involving, in part, the heterochromatic region (result unpublished). DEMEREC, HOLLAENDER, HOULAHAN and BISHOP (1942) working with monochromatic ultraviolet light found in one experiment with 2650 Å, one translocation among 116 treated sperm. No translocations were found in a second such experiment testing 762 treated sperm. Also, using 3130 Å, a test of 1,073 treated sperm failed to find a single translocation.

It has further been demonstrated by KAUFMANN and HOLLAENDER (1946) that if ultraviolet treatment is given to Drosophila males after X-radiation there results a decrease in the expected number of chromosome rearrangements. The reason for this is not clear, but it seems that the ultraviolet may

[1] Presented to the faculty of the graduate school, Indiana University, in partial fulfillment of the requirements for the Doctor of Philosophy Degree in Zoology.

[2] Present address: Department of Zoology, Ohio University, Athens, Ohio.

[3] Contribution No. 545 from the Zoology Department, Indiana University.

cause damage to the points of X-ray breakage such that the broken ends of the chromosomes cannot undergo exchange following fertilization. Or, it may be that the supplementary ultraviolet treatment causes a higher than normal amount of restitution. Similarly, SWANSON (1942, 1944) had found that ultraviolet causes a reduction of the frequency of chromosome changes produced by X-rays in Tradescantia microspores.

Investigations by NOETHLING and STUBBE (1934), and by STADLER and SPRAGUE (1936) on plant material have resulted in abundant evidence that gene mutations are readily induced by ultraviolet light. Further investigations by STADLER (1938), STADLER and UBER (1938, 1942), STADLER and SPRAGUE (1936c), STADLER (1941), and SWANSON (1942, 1944) have indicated, in general, that while chromatid breaks are relatively frequently induced, chromosome breakage with resultant rearrangement is comparatively rare. In their material, most of ends formed by ultraviolet breakage underwent "healing"; that is, they became converted into free ends ("telomeres") incapable of union with other ends. It is true that FABERGÉ has recently (1951) reported abundant chromosome structural changes to be produced by ultraviolet applied to maize pollen, as shown in the resulting endosperm, without consistent differences from the effects of X-rays having appeared, but as yet this work is preliminary, and includes no estimates of the doses compared in terms of their effectiveness in producing point mutations.

It is apparent, therefore, that ultraviolet light is capable of inducing gene mutations in plant and animal material, and although there is abundant evidence of chromatid breakage in plant material, the bulk of the evidence has indicated that rearrangements are more rarely produced than by X-rays producing the same frequency of point mutations or the same frequency of chromatid breaks. It has seemed desirable, however, in view of the apparently conflicting character of some of the evidence to examine the matter further.

It was with this in mind that DR. H. J. MULLER suggested to the writer an investigation of this problem by treatment of adult Drosophila males with a marked Y chromosome, such that males might appear in F_1 as exceptions to expectation due to the loss of the X or Y chromosome because of breakage. As an index of the genetic effectiveness of the ultraviolet treatment the frequency of induced X chromosome lethals was used. In addition, a less extensive investigation was made as to the relative effectiveness of ultraviolet light as opposed to X-rays in the production of chromosome losses under approximately equivalent induction of X chromosome lethal mutations.

MATERIALS AND METHODS

All irradiations were performed in a dark room with the source of the ultraviolet being a Hanovia Utility Model Quartz Lamp (Catalogue #SC-5030-105-125V-60 Cycle A.C.) stabilized by means of a Sola 500 V.A. voltage stabilizer.

To eliminate in so far as possible the considerable amount of visible light emanating from the ultraviolet lamp, a combination of a liquid and a glass filter was employed. The liquid filter, having a 4 cm thickness of a rather con-

centrated solution of nickel and cobalt sulfates (81.89 grams of $NiSO_4 \cdot 6\ H_2O$, and 25 grams of $CoSO_4 \cdot 7\ H_2O$ in 250 ml of distilled H_2O—see BOWEN 1946), was contained within a glass tank having two sides of Corning glass, #791—2 mm thick, so as to permit the transmission of ultraviolet light. A coil of copper tubing through which cold water was circulated throughout the irradiation in order to cool the liquid filter, which would otherwise become heated by the lamp, was also contained within this tank around its periphery. A plate of Corning glass #986 (3 mm thick) was placed on the side of the tank farthest from the lamp and in direct contact with one plate of #791 glass. These two filters in combination removed nearly all of the visible light. A cardboard frame surrounding the filters served to shield out most of the visible light which came around the sides of the filters.

A spectrograph was used to obtain a photograph of the spectral lines produced by this lamp and transmitted by the filters. It was thus determined that the wave lengths 2537 through 3340 Angstrom units were transmitted, the 3130 line being the strongest. DEMEREC and HOLLAENDER (1940) had found the 3130 line to be particularly effective in the induction of mutations in the Drosophila male.

The flies were treated at a distance of 15 cm from the burner unit of the lamp for exposure times of 15 to 60 minutes. Noticeable improvement was observed in survival and fertility in subsequent irradiations. The reason for this is not clear, but it is thought that the glass filters used became more opaque to the shorter wave lengths with further exposure to the intense ultraviolet light. This is thought to be the case because later treatments of supposedly the same or higher dose resulted in better survival and fertility, but about the same frequency of lethals. Thus it would seem that the shorter wave lengths, which cause marked damage to tissues in the adult males but which hardly penetrate to the spermatozoa, have become less intense, while the longer wave lengths, such as 3130, were still being received by the flies at about the same intensity.

The flies to be treated were squeezed, after the method of REUSS (1935), beneath a plate of ultraviolet transmitting glass, Corning #791, after they had been etherized and carefully positioned with their dorsal surfaces down against a layer of cellucotton which in turn overlay a thickness of sponge rubber. In this way the more resistant thorax was protected from damage while the abdomen was flattened considerably. The object of this method was to allow a greater percentage of the incident light to penetrate the ventral body wall of the male fly, and to bring the testes closer to the ventral body wall.

A large number of control males were also so squeezed beneath the same plate of Corning glass but were protected from the mutagenic ultraviolet light by the placing of adhesive cellulose tape over the glass directly above them.

Adult males, not over 8 hours old, of the type $y\ w$ In49 $f \cdot Y^s/y^3 \cdot Y^1$ were used. The Y^s represents the short arm of the Y chromosome attached to the X, and y^3 represents a piece of the left end of the scute-S1 inversional X chromosome bearing a dark yellow allele, the scute-S1 positional allele of scute, the normal allele of achaete, and a section of heterochromatin including

block A, attached to the stump of a Y chromosome consisting of an incomplete short arm and a complete long arm. There were several reasons why males of this genetic type were desirable for treatment with ultraviolet. The Y^s on the X and the portion of the X attached to the Y made these chromosomes longer than a normal X or Y, respectively, thus making them more subject to breakage, if the ultraviolet were capable of such action. The compound of *y* and y^3 gave a lighter than normal (although darker than ordinary yellow) body color so that more of the incident ultraviolet light could penetrate the body tissues, and the presence of the gene for white eye also removes the red pigment from the testis sheath, thus allowing a greater degree of penetration of the testis by the ultraviolet light. The In49 was essential to reduce the frequency of crossing-over in the F_1 females which were tested individually for X chromosome lethals.

Such males were for the most part mated individually after treatment to 3 $ac^3\ w^a\ B \cdot Y^s$ females in a vial. These females were kept in stock with $ac^3\ w^a\ B \cdot Y^s/sc \cdot Y^1$ males. The $sc \cdot Y^1$ is like $y^3 \cdot Y^1$ except in having the normal allele of yellow; thus it bears the normal allele of achaete, so these males do not show achaete. This was desirable for if any non-virgin $ac^3\ w^a\ B \cdot Y^s$ females had been accidentally used, their cross with the males from the stock would not have produced ac^3 males, which (as explained below) would have been classified as exceptions to expectation.

The regular F_1 males from the cross of $y\ w$ In49 $f \cdot Y^s/y^3 \cdot Y^1$ males with $ac^3\ w^a\ B \cdot Y^s$ females should be non-achaete due to the presence of the normal allele of achaete in the $y^3 \cdot Y^1$ chromosomes. It is possible, however, to obtain achaete males in F_1 as exceptions to expectation. Hence, a complete count of all F_1 offspring was made with particular attention being given to the occasional appearance of ac^3 males as exceptions. The mutant achaete[3] is an extreme allele of achaete and causes the absence of the dorso-central bristles, and also the hairs from this region of the thorax.

The crosses involved are given below, the phenotypes being given in parenthesis:

$$P_1: \quad \frac{ac^3\ w^a\ B \cdot Y^s}{ac^3\ w^a\ B \cdot Y^s}\ (ac^3\ w^a\ B) \quad ♀♀ \quad X \quad \frac{y\ w\ \text{In49}/\cdot Y^s}{y^3 \cdot Y^1}\ (y^3\ w\ f) \quad ♂♂$$

$$F_1: \quad \frac{ac^3\ w^a\ B \cdot Y^s}{y\ w\ \text{In49}/\cdot Y^s}\ (w/w^a\ B/B^+) \quad ♀♀ \quad \& \quad \frac{ac^3\ w^a\ B \cdot Y^s}{y^3 \cdot Y^1}\ (w^a\ B) \quad ♂♂ \quad \& \quad \text{occasional } (ac^3\ w^a\ B)\ ♂♂ \text{ occurring as exceptions}$$

$$F_2: \quad \frac{ac^3\ w^a\ B \cdot Y^s}{ac^3\ w^a\ B \cdot Y^s}\ (ac^3\ w^a\ B) \quad ♀♀ \quad \& \quad \frac{ac^3\ w^a\ B \cdot Y^s}{y^3 \cdot Y^1}\ (w^a\ B) \quad ♂♂$$

$$\frac{ac^3\ w^a\ B \cdot Y^s}{y\ w\ \text{In49}/\cdot Y^s}\ (w/w^a\ B/B^+) \quad ♀♀ \quad \& \quad \frac{y\ w\ \text{In49}/\cdot Y^s}{y^3 \cdot Y^1}\ (y^3\ w\ f) \quad ♂♂$$

(In addition, in F_2 there is a low frequency [less than 5%] of single crossovers between the positions of *w* and *B*, beyond In49; phenotypically, these are *ac*^{3} *w*^{a} *B*/*B*$^+$ and *w*/*w*^{a} *B* ♀♀ and *w*^{a} *f* and *y*^{3} *w B* ♂♂.)

A large number of the F_1 females in both treated and control material were tested individually in vials for the presence of lethals in the X chromosome under consideration. Such lethals would be evidenced by an absence of the phenotypically *y*^{3} *w f* males in the F_2 generation. Lethals which were found were made up into stock balanced by a *sc*^{81} *B* InS chromosome, for possible localization and cytological analysis at a later time.

RESULTS AND DISCUSSION OF RESULTS

Frequency of lethals in ultraviolet treated and control material

A total of 30,207 F_1 females, 25,802 F_1 *w*^{a} *B* males, and 44 *ac*^{3} *w*^{a} *B* males were obtained from 23 ultraviolet irradiations. The females thus comprised 53.9% of the total F_1 flies; whereas in control material 14,770 females, 12,783 *w*^{a} *B* males, and 7 *ac*^{3} *w*^{a} *B* males were observed. Here the females comprise 53.6% of the total. Thus it is apparent that dominant lethals have not been induced to an appreciable extent by the ultraviolet treatment.

A total of 10,331 F_1 females from these 23 ultraviolet irradiations were tested for X chromosome lethals, and 124 were found, which gives a frequency of 1.20%.

A total of 2,250 X chromosomes from control material of the same cross were tested for lethals and 3 were found, a frequency of 0.133%. Thus there were over 9 times as many lethals in treated as in control material. The chi-square test of independence on these data gives a chi-square value of 21.048, and hence a probability of less than 0.1% that such a difference could have occurred by chance alone. It is therefore readily apparent that the treated males were exposed to sufficient ultraviolet light to cause appreciable mutagenesis.

A total of 1,752 of the 2,250 X chromosomes from control material which were tested were derived from young males which were virgin at the time they were mated (3 lethals, or 0.17%), while the remaining 498 (0 lethals) were derived from non-virgin young males. The lethal frequencies here are, within the limits of error, the same as found in large scale earlier work (MULLER 1946 and unpublished) in which the mutation frequencies of virgin and non-virgin young males were compared, and found to be about 0.18% and 0.06%, respectively. All the males which were treated with ultraviolet were virgin young males, and thus the chromosomes from treated material which were tested for lethals were all derived from virgin males. It can readily be shown that, considering only the offspring of virgin males, the difference in lethal frequency between the controls (giving 0.17%) and the treated (1.20%) remains highly significant. One thousand and seventy of the control X chromosomes tested were derived from experiments in which the control males were actually squeezed beneath the same plate of glass as the treated males, but the control males were protected from the ultraviolet by the 2 to 3 layers of adhesive cellulose tape previously mentioned.

A summation of all control data, including that obtained from two other stocks in earlier work, gives a total of 22,716 F_1 females, 20,134 F_1 males of the expected type, and 13 males which were exceptions to expectation. The females thus comprised 53.0% of the total F_1 flies, and the exceptional males represented 0.064%. In all, 5,149 X chromosomes were tested in control material and 7 lethals were found, a frequency of 0.136%. The earlier material was all obtained from males which were virgin at the time of mating.

Table 1 presents a summary of all ultraviolet and control results.

TABLE 1

*Summary of all ultraviolet and control results.**

Series	Number of F_1 ♀♀	Number of F_1 $w^a B$ ♂♂	Total number and % of F_1 exceptional ♂♂	Number and % of F_1 sterile exceptional ♂♂	Number of X chromosomes tested	Number of lethals	Percent lethals
Ultra-violet treated	30,207	25,802	44 0.170%	30 0.116%	10,331	124	1.200
Controls from cross of *y w* In49 / · Y* stock	14,770	12,783	7 0.054%	3 0.023%	2,250	3	0.133
Total control data regardless of stock	22,716	20,134	13 0.064%	(not all tested)	5,149	7	0.136

*In addition to the F_1 phenotypes recorded above there were observed, but excluded from this tabulation, a few *y w* / males of non-disjunctional origin and mosaic exceptions such as gynanders and 1/2 achaete males.

Discussion of genetic evidence for ultraviolet induced chromosome breakage

Forty-four males among 25,846 F_1 males from treated material were found to be $ac^3 w^a B$ and hence exceptions to expectation. These exceptional males thus comprised 0.170% ± 0.026% of the total F_1 males. In control material on this same stock there were 7 such exceptional males among a total of 12,790 F_1 males, or a frequency of 0.054% ± 0.020%. The difference, 0.116% ± 0.033%, is clearly significant. Similarly, the difference in frequency of sterile males, considered by themselves, is clearly significant.

It is necessary at this point to discuss the various mechanisms whereby achaete males can appear in F_1. There are at least 9 possible explanations for the appearance of an achaete male in F_1. Ultraviolet treatment could conceivably play a role in 7 of these.

The four mechanisms which could give rise to sterile males will be considered first. If the ultraviolet treatment in some way damaged either the X or Y chromosome so as to cause the loss of the whole chromosome in later mitotic divisions following fertilization, a sterile male would result. This dam-

age might possibly be (1) a breakage of the X or Y before its duplication, with sister chromatids undergoing union of broken ends following duplication, so as to give acentric and dicentric chromosomes which would become lost upon the mitotic spindle following fertilization. Or (2) it might be that no actual break is involved, but rather that as a result of treatment the chromatids simply lag behind on the spindle of the first zygotic division so as to become shut out of both daughter nuclei by the formation of the nuclear membranes. Damage to the centromere might possibly explain such a loss due to lagging. Also (3) if breaks were induced in the 2 arms of the $y^3 \cdot Y^1$ chromosome, such that the normal allele of achaete^3 in the one arm, and one or more of the fertility genes in the other arm were lost, with the subsequent union of the broken ends of the two stumps so as to form a ring Y chromosome, there could result a sterile achaete male. These three methods could give sterile achaete males due to treatment. Related to this (3a) would be two breaks in the X, one near each end, with deletion of the long interstitial portion, and union of the two terminal pieces with each other; in this case, however, the left break would have to be in the very small region to the left of the achaete locus if the offspring are to show achaete, and there is only a very small likelihood of its being so placed.

It would be possible, however, to obtain such sterile exceptional males by (4) a process upon which ultraviolet irradiation could have no effect. If, prior to irradiation, at a time when the X and Y chromosomes are undergoing their meiotic (or even pre-meiotic) divisions, non-disjunction or lagging of one or both of the X and Y chromosomes, or their chromatids, were to occur, then there could result sperm bearing neither an X nor a Y which upon fertilizing an egg with the $ac^3\ w^a\ B \cdot Y^s$ X chromosome would give rise to a sterile achaete male indistinguishable from those which might have appeared due to loss of the X or Y chromosome, or the formation of a ring-shaped Y, because of treatment. However, such males caused by aberrant meiotic or pre-meiotic divisions should occur as frequently in control as in treated material so that an observed difference in the frequency of such sterile males in control and treated material should be due solely to the loss of the X or Y chromosome because of treatment.

There are five possible means, which shall be numbered 5 to 9, of obtaining fertile achaete males. (5) If the locus of the normal allele of achaete is deleted as an interstitial deletion of the portion of the chromosome bearing this locus, (6) as a terminal deletion of the entire y^3-containing portion of $y^3 \cdot Y^1$, with healing of the broken end of the stump such as occurs in some broken plant chromosomes, or (7) by the formation of a ring-shaped Y which causes the terminal deletion of the achaete$^+$ locus from the "left" arm of $y^3 \cdot Y^1$, but not the loss of any of the fertility genes from the other arm, there would result a fertile achaete male. Similarly, (8) if the normal allele of achaete^3 in $y^3 \cdot Y^1$ were to undergo a gene mutation to achaete then again there would result a fertile achaete male. Ultraviolet treatment could conceivably influence the frequency of these four possible mechanisms for the production

of fertile achaete males. Fertile achaete males could also result (9) from a rare crossover in the male between the X and Y chromosomes during or previously to meiosis. Such a process could give rise to some sperm bearing a complete Y chromosome and such sperm upon fertilizing an egg with the *ac*^{3} *w*^{a} *B* · Y^s X chromosome would produce a fertile achaete male. In this material, ultraviolet light could not produce fertile achaete males by this method, since it acted only upon postmeiotic stages of the sperm from which the offspring studied were derived.

By means of a special test cross it was possible to narrow down the means by which a given fertile male had appeared. The fertile exceptional F_1 male in question was mated to virgin females of the type *sc*^{J1} *pn w rb cm ct*/ *y sc*^{8} *f* In49 *v w*^{a}. Then, depending on the types of males appearing in the next generation (to be designated as F_2), and their fertility or sterility, it was possible to designate the reason for the fertility and exceptionality as due to one of the following 3 causes: the presence of a complete Y (9), the loss by deletion of the whole *y*^{3}-containing region (5, 6, or 7), or mutation restricted to the immediate neighborhood of the achaete$^+$ locus in the *y*^{3} · Y^1 chromosome (8). If the exceptional F_1 male were fertile due to its possession of a complete Y, then only the *y sc*^{8} *f* In49 *v w*^{a}/Y F_2 males would appear since the lethal involved in *sc*^{J1} (to the left of it as represented in a standard map) would not be covered by a normal Y; these F_2 males would, because of the complete Y, be fertile. If the fertility of the F_1 male were due to deletion or loss of the whole achaete$^+$ region, however, then again only the *y sc*^{8} *f* In49 *v w*^{a} males would appear in F_1, but these would all be sterile due to the lack of Y^s, since this is not present on their X chromosome. And if the fertile achaete F_1 male were due to mutation or loss restricted to the achaete$^+$ locus or its immediate vicinity in *y*^{3} · Y^1, then both *sc*^{J1} *pn w rb cm ct* and *y sc*^{8} *f* In49 *v w*^{a} males would appear in F_1 since the Y chromosome would cover the lethal in the *sc*^{J1} chromosome. Both of these male types would, however, be sterile for the Y^s would again be absent.

The results indicate that with the dosages administered some degree of chromosome loss must have been induced, although it is not possible to be sure that this was due to actual chromosome breakage. Of the 44 achaete^3 males among a total of 25,846 males obtained in F_1 from treated males, 30 were found to be sterile; while in the controls there were 3 such sterile males among 7 achaete^3 males in a total of 12,790 F_1 males. A chi-square test of independence performed on these data for the appearance of sterile males gives a value of 8.592 for chi-square, and hence a probability of less than one per cent that such a difference could have occurred by chance alone. This of course assumes that no large proportion of the F_1 males recorded as sterile were potentially fertile males which died before they could breed or which were with infertile females. As for this possibility each male tested was mated to several virgin females and the great majority of the males survived sufficiently long (several days) after mating that there could be little doubt as to whether they were really sterile.

The significant excess of sterile males in the treated material must have been caused by mechanisms (1) to (3), i.e., single breakage followed by loss due to acentric and dicentric chromosome formation, or lagging, or double breakage followed by ring formation. However, if double breakage (3) occurred, then single breakage (1) followed by loss should have been much more frequent. And as for lagging (2), we might expect this often to apply to just one chromatid, leading to ½ ac^3 males and ½ ac^3 sex mosaics. Four ½ ac^3 males and three ½ ac^3 sex mosaics were observed which were probably due to the loss, by lagging, of the $y^3 \cdot Y^1$ or the $y\,w$ In49 $f \cdot Y^s$ chromosome from some cells. In addition to these there were 7 other mosaic flies obtained in treated material which were due either to loss, by lagging, of the $ac^3\,w^a\,B \cdot Y^s$ chromosome (which could not have been caused by treatment) or were of uncertain origin. In control material only one sex mosaic was observed and no ½ ac^3 males were observed. The sterile males were, therefore, probably produced through single breakage (1) or possibly lagging (2) of the X or $y^3 \cdot Y^1$ chromosome.

In order to obtain further evidence as to whether the losses of the entire Y had been caused primarily by breakage or by lagging it had been planned to carry out a similar experiment with ring X chromosomes since on the breakage but not on the lagging mechanism rings would be lost much oftener than rod-shaped chromosomes. However, before this was undertaken, an experiment was reported by FABERGÉ and MOHLER (1952), giving evidence of an especially high frequency of losses of ring X chromosomes treated with ultraviolet in the spermatozoon stage of Drosophila. In this work the losses for which evidence was sought were those lethal to the zygote, as shown by the reduction in the frequency of females in the offspring in comparison with males, but as previous work has shown production of these is accompanied by that of " viable losses " such as those found as sterile exceptional males in our own work. It is unfortunate that the frequency with which breakage losses are to be expected for a given mutagenic dose of ultraviolet cannot be calculated from their data, so as to be applied to ours, since they do not report tests of the frequency of induced lethals. However, their work, taken in conjunction with ours, makes it highly probable that most of our sterile exceptional males were caused by chromosome breakage induced by the ultraviolet.

Twelve of the 44 exceptional F_1 males in treated material were found to be fertile. (Two of the 44 were not tested for fertility.) Eight of these fertile males gave a positive test for the presence of a complete Y chromosome and therefore could not have been caused by treatment (mechanism 9). Three proved to have deletion of the achaete$^+$ region in $y^3 \cdot Y^1$ (mechanism 5, 6 or 7). One proved to have mutation or loss of the achaete$^+$ locus (and possibly of a minute region next to it) in the $y^3 \cdot Y^1$ chromosome (8). This could not be further tested since by the time that it had been determined that a mutation or loss of the achaete$^+$ locus may have occurred, all the males were sterile due to the fact that their X chromosome did not carry Y^s.

Two of the fertile achaete males in the controls gave positive tests for the

presence of a complete Y chromosome (9), while 2 gave a positive test for deletion of the region including the achaete$^+$ locus (5, 6 or 7). The difference in number of fertile achaete males obtained in treated and control series due to these causes is not sufficiently great to be significant.

It is of interest to note that only three fertile males which gave positive tests for deletion of the achaete locus were found in the treated material, while two such males were also found among the smaller number of control males. Thus it is apparent that the ultraviolet treatment has not caused an appreciable amount of interstitial deletion, involving two breaks near together. Neither has it caused double breakage (1 break in each arm) of the Y, in such a distal position as to retain all the fertility genes of the Y^1 arm, followed by ring formation, although this process has been reported to result from X-irradiation, as have other rearrangements of the Y in which one break occurred distally to all fertility genes.

Finally, it is also apparent that the ultraviolet has not caused an appreciable amount of single breakage of the $y^3 \cdot Y^1$ chromosome with "healing," so as to form surviving chromosomes with a terminal deletion, thus lacking y^3. Hence the single breakage induced by ultraviolet must, at least in the great majority of cases, be followed by union of broken ends. That is, an interstitial gene does not automatically acquire the property of a telomere in this material. This last result is in contrast to the situation found in plant material by STADLER and UBER where they found breakage followed by formation of "healed" terminal deletions to be much more freely induced by ultraviolet light than breakage followed by union of broken ends.

Comparison of the production of F_1 sterile exceptional males by X-rays and ultraviolet light at nearly equivalent induced lethal frequency

Three X-ray irradiations were made of $y\,w$ In49 $f \cdot Y^s/y^3 \cdot Y^1$ males in which a low dose was administered so as to give a lethal frequency approximately equivalent to that obtained with ultraviolet. This was done so that some idea could be obtained as to the relative effectiveness of ultraviolet versus

TABLE 2

Comparison of X-ray, ultraviolet, and control results.

Dosage	Number F_1 ♀♀ ++	Number F_1 $w^a\,B$ ♂♂	Number F_1 $ac^3\,w^a\,B$ ♂♂	Percent sterile $ac^3\,w^a\,B$ ♂♂	Number X chromosomes tested	Number lethal	Percent lethal
876 r	2,558	2,314	9 (8 ster.)	0.344	500	11	2.20
584 r	3,542	3,227	9 (7 ster.)	0.216	491	8	1.63
365 r	3,537	3,344	8 (5 ster.)	0.149	495	6	1.21
Ultra-violet	30,207	25,802	44 (30 ster.)	0.116	10,331	124	1.20
Control from virgin ♂♂	10,830	9,295	5 (3 ster.)	0.032	1,752	3	0.171
Total control	14,770	12,783	7 (3 ster.)	0.023	2,250	3	0.133

X-rays in the production of sterile exceptional males in F_1 at equivalent lethal frequencies. X-radiations of approximately 876 r, 584 r, and 365 r units were given. The results from these three treatments are given in table 2, together with the results obtained from ultraviolet treatment, and from control material.

All of the data presented in table 2 were derived from virgin F_1 males except for a portion of the control data, which have been presented in two parts.

1. The standard errors of the above frequencies can be determined by use of the formula: $\sigma = \sqrt{pq/N}$, where p is equal to the frequency of lethal-bearing chromosomes, and q the frequency of non-lethal-bearing chromosomes, and N is the total number of X chromosomes tested. By this method the following lethal frequencies (induced plus control) and their standard errors are obtained:

876 r	$2.20\% \pm 0.656\%$
584 r	$1.63\% \pm 0.566\%$
365 r	$1.21\% \pm 0.489\%$
U. V.	$1.20\% \pm 0.107\%$
Control from virgin ♂♂	$0.17\% \pm 0.098\%$

The control lethal frequency, 0.17%, derived from virgin P_1 males is subtracted from the frequencies obtained with the treatments to give the induced lethal frequency in each case; and the standard errors of these induced lethal frequencies are determined for each case by calculating the square root of the sum of the squares of the standard error for that treatment, and for the control frequency. In this way the induced lethal frequencies and their standard errors are determined to be as follows:

876 r	$2.03\% \pm 0.663\%$
584 r	$1.46\% \pm 0.574\%$
365 r	$1.04\% \pm 0.499\%$
U. V.	$1.03\% \pm 0.145\%$

The above X-ray dosages are related to one another as 3 : 2 : 1.25. The induced frequency of lethals, therefore, for a dose " 1 " can be obtained from each of the above frequencies by dividing the induced lethal frequency and its standard error by the appropriate number: 3, 2, or 1.25. The following induced frequencies of lethals for a dose " 1 " are thus obtained:

$0.677\% \pm 0.221\%$
$0.730\% \pm 0.287\%$
$0.832\% \pm 0.399\%$

It is desirable to determine a weighted mean of the above frequencies so that the frequencies with the smaller standard errors play a heavier role. The appropriate weight for each case is obtained by taking the reciprocal of the square of the standard error. The induced lethal rates and their standard errors for a dose " 1 " are then multiplied by these weights to give weighted

values for the induced frequencies for a dose " 1." The sum of the weighted values is then divided by the sum of the weights to give the weighted mean frequency for a dose " 1." The weighted mean standard error is determined by dividing the square root of the sum of the squares of the weighted standard errors by the sum of the weights. The weighted mean frequency of induced lethals for a dose " 1," representing 292 r, is thus found to be: 0.715% ± 0.160%. This agrees well, considering its error, with the value expected on the basis of previous work of others.

It was found above that the induced frequency of lethals from ultraviolet treatments was 1.03% ± 0.145%. This is 1.4405 times the above weighted mean frequency of induced lethals for an X-ray dose of 292 r, and hence is the frequency obtained with 420.64 r of X-rays, as calculated from our results.

2. Methods exactly like those outlined above were used to determine the mean frequency of X-ray induced sterile exceptional males in F_1 for a dose " 1." The steps involved are given below without accompanying explanations as to the procedure since that was given above.

a. Frequencies (induced plus control) of sterile exceptional males in F_1:

876 r	0.344% ± 0.121%
584 r	0.216% ± 0.082%
365 r	0.149% ± 0.066%
U. V.	0.116% ± 0.021%
Control from virgin ♂♂	0.032% ± 0.0185%

b. Induced frequencies of sterile exceptional F_1 males:

876 r	0.312% ± 0.123%
584 r	0.184% ± 0.084%
365 r	0.117% ± 0.068%
U. V.	0.084% ± 0.028%

c. X-ray induced frequencies of sterile exceptional males for a dose " 1 ":

0.104% ± 0.041%
0.092% ± 0.042%
0.094% ± 0.054%

d. The above standard errors for a dose " 1 " are then utilized in the determination of appropriate weights as was done in determining the weighted mean frequency of induced lethals for a dose " 1." The weighted mean frequency of induced sterile exceptional males for a dose " 1 " is thus determined to be 0.097% ± 0.026%.

3. It is now possible to compare the ultraviolet and X-ray results, at equivalent induced lethal frequencies, as to the frequencies of sterile exceptional males in F_1. It was found above that if the weighted mean frequency of X-ray induced lethals for a dose of 292 r is multiplied by 1.4405, a frequency of induced lethals is obtained which is equivalent to that obtained by ultraviolet treatment (1.03% ± 0.145%). And since the frequency of induced

sterile exceptional males is proportional to the frequency of induced lethals, it is possible to multiply the weighted mean frequency of induced sterile exceptional F_1 males for a dose of 292 r by the same figure 1.4405, to obtain the frequency of sterile exceptional males which would be expected with an X-ray dose which gave 1.03% lethals. Therefore, $0.097\% \pm 0.026\% \times 1.4405 = 0.140\% \pm 0.037\%$ is this expected frequency. The question is, is this frequency significantly different from that obtained by ultraviolet treatment, $0.084\% \pm 0.028\%$? The actual difference between these values is 0.056% and the standard error of the difference is 0.0464%. A value of 1.217 is thus obtained by dividing the difference by the standard error of the difference.

This difference, therefore, does not have a high degree of significance. A difference of this size, however, in this "expected" direction would be expected to occur by chance in only 1 sample out of 9 of the same kind. It does, therefore, become probable that ultraviolet does not induce as many chromosome losses as a dose of X-rays having the same lethal-inducing effectiveness.

One of the 26 exceptional males obtained in the X-ray treatments was found to be a fertile achaete exception due to deletion of the whole achaete$^+$ region in the $y^3 \cdot Y^1$ chromosome. This then is one male among a total of 8,911 F_1 males, or $0.011\% \pm 0.011\%$. Among the F_1 males from ultraviolet treatments, there were three such males among 25,846, or a frequency of $0.012\% \pm 0.007\%$. The difference is not significant.

The above results (though inconclusive) indicating fewer whole chromosome losses (single breaks) induced by ultraviolet light than by a nearly equivalent dosage of X-rays are strengthened by the cytogenetic analysis of a number of lethals induced by ultraviolet on the one hand and by a low dosage of X-rays on the other hand. VALENCIA and MCQUATE (1951) found that among 70 ultraviolet lethals, induced by a dosage giving 1.3% lethals, there were three cytologically visible chromosome rearrangements: 1 translocation, 1 inversion, and 1 deletion. VALENCIA (1952) reported that 77 X-ray lethals, produced by a dose that had given 1.8% lethals, included 12 chromosome changes: 7 translocations, 4 inversions, and 1 deletion.

SUMMARY

1. Young virgin males with a marked Y chromosome, $y^3 \cdot Y^1$, were treated with filtered ultraviolet light (ranging from 2537 to 3340 Å) from a Hanovia lamp, and mated to females containing achaete^3. As an index of the treatment's genetic effectiveness, F_1 females were tested for X chromosome lethals. One hundred and twenty-four lethals were detected in 10,331 treated chromosomes (1.20%); in the control material there were 3 lethals in 2,250 chromosomes (0.133%). The probability that such a difference could be due to chance is less than one in one thousand.

2. The regular males in the F_1 do not show ac^3 since $y^3 \cdot Y^1$ contains the normal allele of achaete. In a total of 25,846 F_1 males, however, 30 achaete exceptions were obtained which were sterile, while there were only 3 sterile

achaete exceptions among 12,790 control F_1 males. This difference has less than 1% probability of occurrence by chance. Such males represent the loss of the paternal X or all or part of both arms of $y^3 \cdot Y^1$. This must have been caused by breakage leading to acentric and dicentric chromosomes, or by lagging, or by the formation of a ring-shaped Y chromosome following double breakage. Reasons are given for concluding that the first of these three interpretations is the correct one for the great majority of the cases.

3. There are also mechanisms whereby fertile achaete males could appear in F_1 as the result of interstitial deletion, ring chromosome formation, or terminal deletion of ac^+ in $y^3 \cdot Y^1$; but in the treated material only three such males were found, while two were also found in the controls (approximately 0.01% in both cases). The ultraviolet light, therefore, has not caused an appreciable amount of interstitial deletion, involving 2 breaks near together, nor has it caused double breakage (1 break in each arm) of the Y in such a distal position as to retain all the fertility genes of the Y^1 arm, followed by ring formation. Finally it is also evident that ultraviolet has not caused single breakage of the $y^3 \cdot Y^1$ chromosome with "healing," so as to form surviving chromosomes with a terminal deletion, thus lacking y^3. Hence, the single breakage which appears to be induced by ultraviolet would in the great majority of cases be followed by union of broken ends. That is, an interstitial gene which has become disconnected by the action of ultraviolet does not ordinarily acquire the property of a telomere in this material.

4. Data were obtained which made possible a comparison of the relative effectiveness of ultraviolet and X-rays in the production of sterile achaete males at an equivalent lethal frequency. At an equivalent induced frequency of 1.03% lethals, ultraviolet induced $0.084\% \pm 0.028\%$ of sterile exceptional males, while X-rays induced a mean frequency of $0.140\% \pm 0.037\%$. The difference, 0.056%, divided by its standard error, 0.046%, is about 1.2. This difference, therefore, does not have a high degree of significance. A difference of this magnitude and in this direction, however, would be expected to occur by chance in only about 1 sample out of 9 of the same kind. It does, therefore, become probable that ultraviolet does not induce as many chromosome losses as a dose of X-rays having the same lethal-inducing effectiveness. The results of the writer and VALENCIA, on the frequency of chromosome changes found among lethals induced by comparable doses of ultraviolet and X-rays have given evidence for the same conclusion.

5. On the other hand, the frequency of breakage produced by ultraviolet in Drosophila spermatozoa appears, on the basis of both the results herein reported and of those in the works of the writer and VALENCIA, to be at least half as great as that produced by X-rays giving the same frequency of gene mutations. Hence the two agents are more nearly equivalent, mutagenically, than had usually been supposed.

ACKNOWLEDGMENTS

The author wishes to express his appreciation to DR. H. J. MULLER for suggesting this problem, for the design of the stocks used, and for many help-

ful suggestions and criticisms; and also to DR. HELEN UNGER MEYER and DR. J. I. VALENCIA for their interest and suggestions.

A part of the work was done while a recipient of an Indiana University Department of Zoology Eigenmann Fellowship.

The work was aided by grants to DR. MULLER from the United States Public Health Service on the recommendation of the National Advisory Cancer Council.

LITERATURE CITED

ALTENBURG, E., 1930 The effect of ultraviolet radiation on mutation. Anat. Rec. **47:** 383.

1931 Genetic effects of ultraviolet radiation. Anat. Rec. **51:** (Supp.), 108–109.

1933 The production of mutations by ultraviolet light. Science **78:** 587.

1934 The artificial production of mutations by ultraviolet light. Amer. Nat. **68:** 491–507.

1936 The production of mutations by the polar cap method of treatment. Biol. Zh. **5:** 27–34.

BOWEN, E. J., 1946 The Chemical Aspects of Light. Clarendon Press, Oxford.

DEMEREC, M., and A. HOLLAENDER, 1940 Yearb. Carnegie Institution **39.**

DEMEREC, M., A. HOLLAENDER, M. B. HOULAHAN and M. BISHOP, 1942 Effect of monochromatic ultraviolet radiation on *Drosophila melanogaster*. Genetics **27:** 139–140.

FABERGÉ, A. C., 1951 Ultraviolet induced chromosome aberrations in maize. Genetics **36:** 549–550.

FABERGÉ, A. C., and J. D. MOHLER, 1952 Breakage of chromosomes produced by ultraviolet radiation in *Drosophila*. Nature **169:** 278–279.

KAUFMANN, B. P., and A. HOLLAENDER, 1946 Modification of the frequency of chromosomal rearrangements induced by X-rays in *Drosophila*. II. Use of ultraviolet radiation. Genetics **31:** 368–376.

MACKENZIE, K., and H. J. MULLER, 1940 Mutation effects of ultraviolet light in *Drosophila*. Proc. Roy. Soc. Edinb. B, **129:** 494–517.

MCQUATE, J. T., 1950 Chromosome loss occasioned by ultraviolet treatment of *Drosophila* spermatozoa. (Abstr.) Genetics **35:** 680–681.

MULLER, H. J., 1927 Artificial transmutation of the gene. Science **66:** 84–87.

1941 Induced mutations in *Drosophila*. Cold Spring Harbor Symp. Quant. Biol. **9:** 151–165.

1946 Age in relation to the frequency of spontaneous mutations in *Drosophila*. Year Book of Am. Philos. Soc. 1945, 150–153.

1954 The Manner of Production of Mutations by Radiation (see Section 10), Radiation Biology, Vol. I, Chap. 7, Edited by A. Hollaender. The McGraw-Hill Book Co., New York.

MULLER, H. J., and K. MACKENZIE, 1939 Discriminatory effect of ultraviolet rays on mutation in *Drosophila*. Nature **143:** 83.

NOETHLING, W., and H. STUBBE, 1934 Die Auslösung von Genmutationen nach Bestrahlung reifer männlicher Gonen mit Licht. Zeit. ind. Abst. Ver. **67:** 152–172.

REUSS, A., 1935 Über die Auslösung von Mutationen durch Bestrahlung erwachsener *Drosophila* Männchen mit ultraviolettem Licht. Zeit. ind. Abst. Ver. **70:** 523–525.

SLIZYNSKI, B. M., 1942 Deficiency effects of ultraviolet light in *Drosophila melanogaster*. Proc. Roy. Soc. Edinb. B, **61:** 297–315.

STADLER, L. J., 1938 On the possibility of disentangling by spectrological means the complex of genetic effects induced by radiations. Genetics **23:** 170.

1941 The comparison of ultraviolet and X-ray effects on mutation. Cold Spring Harbor Symp. Quant. Biol. **9:** 168–178.

STADLER, L. J., and G. F. SPRAGUE, 1936 Genetic effects of ultraviolet radiation in maize. Proc. Nat. Acad. Sci. Wash. **22:** 572–591.

STADLER, L J., and F. M. UBER, 1938 Preliminary data on genetic effects of monochromatic ultraviolet radiation in maize. Genetics **23**: 171.

1942 Genetic effects of ultraviolet radiation in maize. IV. Comparison of monochromatic radiations. Genetics **27**: 84–118.

SWANSON, G. P., 1942 The effects of ultraviolet and X-ray treatment on the pollen tube chromosomes of Tradescantia. Genetics **27**: 491–503.

1944 X-ray and ultraviolet studies on pollen tube chromosomes. I. The effect of ultraviolet (2537 Å) on X-ray induced chromosomal aberrations. Genetics **29**: 61–68.

VALENCIA, J. I., 1952 Chromosome changes in *Drosophila melanogaster* spermatozoa induced by genetically comparable doses of X-rays and ultraviolet. (Abstr.) Genetics **37**: 633.

VALENCIA, J. I., and J. T. MCQUATE, 1951 A cytogenetic analysis of 70 ultraviolet-induced X chromosome lethals in *Drosophila*. (Abstr.) Genetics **36**: 580–581.

INFORMATION FOR CONTRIBUTORS

Contributions to GENETICS may be in the field of genetics proper, or in any scientific field, if of such a character as to be of primary genetic interest.

The length of manuscripts will be limited to twenty printed pages, including tables and illustrations. Longer papers may be published by special vote of the Editorial Board and if the author agrees to bear the cost of the additional pages. In case unusually extensive tabular material cannot be printed, it will be kept on file on request, provided two copies are furnished by the author.

The *Galton and Mendel Memorial Fund* established in 1923 from donations of biologists and other persons interested in the progress of biological discovery is available to be applied toward the cost of reproducing illustrations and of printing expensive tables and formulae.

Authors should submit two copies of their manuscripts to the Editors. Manuscripts should conform to the general usage in GENETICS, particularly in regard to references to literature, arrangement of "Literature Cited" and inclusion of a summary. In case of "Acknowledgments," these should follow the "Summary." Footnotes should be avoided in the text whenever possible and their content be inserted, in parentheses, after the appropriate sentence. Footnotes to tables should be marked with an asterisk, dagger, or other symbol so as not to be confused with the figures in tables. Legends for figures and plates should be type-written separately from the illustrations. In addition to the original illustrations photographic copies should accompany the manuscript. Tables also should be type-written separately. The metric system should be used whenever possible.

Galley proofs and page proofs will be sent to authors, except in case of authors resident overseas who, in general, will receive galley proofs only. Authors should leave forwarding directions whenever they are to be away from the address sent with the manuscript.

Reprints may be ordered at the time galley proof is returned. The author will be billed for them in accordance with the schedule of charges appearing on the order blank. Copies of the schedule may be obtained from the Editorial Office.

Manuscripts and editorial correspondence should be addressed to the Editors of GENETICS, Genetics Building, University of Wisconsin, Madison 6, Wisconsin.

GENETICS Vol. 39 No. 4 JULY, 1954

CONTENTS OF PRECEDING ISSUES

NOVEMBER, 1953

GOWEN, JOHN W., JANICE STADLER, H. H. PLOUGH and HELEN N. MILLER, Virulence and immunizing capacity of *Salmonella typhimurium* as related to mutations in metabolic requirements.

SIEGEL, R. W., A genetic analysis of the mate-killer trait in *Paramecium aurelia*, variety 8.

LEVINE, MYRON, The diverse mate-killers of *Paramecium aurelia*, variety 8: their interrelations and genetic basis.

MCCLINTOCK, BARBARA, Induction of instability at selected loci in maize.

STEBBINS, G. L., and FUNG TING PUN, Artificial and natural hybrids in the Gramineae, tribe Hordeae. VI. Chromosome pairing in *Secale cereale* × *Agropyron intermedium* and the problem of genome homologies in the Triticinae.

EATON, ORSON N., Heterosis in the performance of mice.

KAPLAN, WILLIAM D., The influence of Minutes upon somatic crossing over in *Drosophila melanogaster*.

Abstracts of papers presented at the 1953 Meetings of the Genetics Society of America.

JANUARY, 1954

PLOUGH, HAROLD H., Edwin Grant Conklin.

STALKER, HARRISON D., Parthenogenesis in Drosophila.

CASTLE, W. E., Coat color inheritance in horses and in other mammals.

SLATIS, HERMAN M., and DAVID A. WILLEMET, The miniature complex in *Drosophila melanogaster*.

SWAMINATHAN, M. S., Nature of polyploidy in some 48-chromosome species of the genus Solanum, section Tuberarium.

BRNCIC, DANKO, Heterosis and the integration of the genotype in geographic populations of *Drosophila pseudoobscura*.

CAIN, A. J., and P. M. SHEPPARD, Natural selection in Cepaea.

AVERS, CHARLOTTE J., Chromosome behavior in fertile triploid Aster hybrids.

NOVITSKI, EDWARD, The compound X chromosomes in Drosophila.

MARCH, 1954

SCHERR, GEORGE H., MARVIN FISHMAN and R. H. WEAVER, The mutagenicity of some carcinogenic compounds for *Escherichia coli*.

BENNETT, J. H., Panmixia with tetrasomic and hexasomic inheritance.

KRAMER, H. H., ROSEMARIE VEYL and W. D. HANSON, The association of two genetic linkage groups in barley with one chromosome.

LEVINTHAL, CYRUS, Recombination in phage T2: its relationship to heterozygosis and growth.

KALTER, HAROLD, The inheritance of susceptibility to the teratogenic action of cortisone in mice.

NOVITSKI, E., and G. BRAVER, An analysis of crossing over within a heterozygous inversion in *Drosophila melanogaster*.

WALKER, B. E., and E. R. BOOTHROYD, Chromosome numbers in somatic tissues of mouse and man.

STEFFENSEN, DALE, and T. J. ARNASON, Frequency of chromosome aberrations produced by fractional doses of X-rays in Tradescantia.

HANSON, EARL D., Studies on kappa-like particles in sensitives of *Paramecium aurelia*, variety 4.

CALDECOTT, RICHARD S., B. H. BEARD and C. O. GARDNER, Cytogenetic effects of X-ray and thermal neutron irradiation on seeds of barley.

MAY, 1954

BROWMAN, LUDVIG G., Microphthalmia and maternal effect in the white rat.

SMITH, WILLIAM K., Viability of interspecific hybrids in Melilotus.

KIMURA, MOTOO, Process leading to quasi-fixation of genes in natural populations due to random fluctuation of selection intensities.

RUBIN, B. A., The quantitative estimation of radiation induced mutations to streptomycin resistance in *Escherichia coli*.

BREWBAKER, JAMES L., Incompatibility in autotetraploid *Trifolium repens* L. I. Competition and self-compatibility.

WHITING, ANNA R., and ROBERT C. VON BORSTEL, Dominant lethal and inactivation effects of nitrogen mustard on Habrobracon sperm.

PITTENGER, THAD H., The general incidence of pseudo-wild types in *Neurospora crassa*.

POMPER, S., K. M. DANIELS and D. W. MCKEE, Genetic analysis of polyploid yeast.

WOLFF, SHELDON, Some aspects of the chemical protection against radiation damage to *Vicia faba* chromosomes.

SANDLER, L., and G. BRAVER, The meiotic loss of unpaired chromosomes in *Drosophila melanogaster*.

STEBBINS, G. L., and ANTERO VAARAMA, Artificial and natural hybrids in the Gramineae, tribe Hordeae. VII. Hybrids and allopolyploids between *Elymus glaucus* and Sitanion spp.

RENDEL, JAN, A. NEIMANN SORENSEN and M. R. IRWIN, Evidence for epistatic action of genes for antigenic substances in sheep.

ALEXANDER, MARY L., Mutation rates at specific autosomal loci in the mature and immature germ cells of *Drosophila melanogaster*.

SINGLE NUMBERS $1.50 ANNUAL SUBSCRIPTION $8.00

obtainable from

GENETICS, INC., BUSINESS OFFICE, MT. ROYAL AND GUILFORD AVENUES, BALTIMORE 2, MARYLAND